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RESEARCH NOTE

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Effects of Fermented *Ssanghwatang* on Swimming Capacity in Mice

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Abstract Swimming enhancing effects of *ssanghwatang* fermented with *Saccharomyces cerevisae* in mice were investigated, and then the blood biochemical parameters related to fatigue were measured. Compared to the control group, the swimming time was significantly increased in fermented *ssanghwatang* (FST) group from day 4 (29.27±4.73 vs. 50.27±9.05 min, $p<0.05$). The lactate level, an important indicator of fatigue, was drastically lower in FST group than in control group (2.13±0.13 vs. 1.40±0.12 mmole/L, $p<0.01$). These results suggest that FST possesses stimulatory effects which can enhance exercise performance and reduce fatigue.

Keywords: fermented *ssanghwatang*, swimming capacity, anti-fatigue

Introduction

Ssanghwatang is a traditional Chinese herbal medicine formula consisting of 10 ingredients (*Paeonia iactiflora* Pall (10.0 g), *Rehmannia glutinosa* Liboschitz (4.0 g), *Astragalus membranaceus* Bunge (4.0 g), *Astragalus membranaceus* Nakai (4.0 g), *Cnidium officinale* Makino (4.0 g), *Cinnamomum cassia* Presl (4.0 g), *Glycyrrhiza uralensis* Fisch (4.0 g), *Zingiber officinale* Rosc. (4.0 g), *Ziziyphus jujube* Mill. var inermis Ddhder (4.0 g), *Cervus nippon* Temminck (4.0 g) (1). It has long been used for the development of physical strength, the inhibition of inflammation, the relief of pain, and the reduction of fatigue. *Ssanghwatang* has also been clinically reported to relieve chronic fatigue syndrome and has been widely used as a nutritional tonic in commercial medical products in many countries (1). Traditional medicines are being extensively studied for their physiologically active agents, which may be of clinical significance. For example, traditional medicines with anti-fatigue activity and improving immobility behavior have been researched in the forced swimming test (2-5).

Fermentation is generally performed by useful probiotics such as *Bifidobacterium* sp. and *Saccharomyces* sp., which transform some components of food to functional compounds (6,7) as well as the production of amino acids, vitamins, and the organic acids (8,9). Therefore, fermented *ssanghwatang* (FST) may be used as a new medical tonic than unfermented *ssanghwatang*. However, no detailed studies have ever been performed to examine whether FST reduces fatigue *in vivo*.

Matsumoto *et al.* (10) developed an adjustable current water pool to evaluate the exercise capacity of mice treated with different diets or drugs. The swimming capacity test resolves various issues with other exercise tests and allows for reliable and reproducible evaluation of the physical work capacity on mice.

In the present study, the stimulatory effects of FST on swimming capacity and anti-fatigue in mice were investigated using an adjustable-current water pool.

Materials and Methods

Preparation of fermented *ssanghwatang* *Ssanghwa* extract (15°Bx) was treated with α-glucosidase (glucoamylase) at 50°C for 15 min and then the denatuation of enzyme was carried out (90°C, 10 min). The *ssanghwa* extract was filtered and fermented at 35°C for 18 hr with *Saccharomyces cerevisae* (0.02%, w/v). The fermented *ssanghwatang* (FST) was then sterilized, concentrated (67°Bx) and lyophilized. The lyophilized FST powder was kept at −20°C until needed.

Animals and diets Male ICR mice (5 weeks) were purchased from Orient Bio (Seongnam, Korea) and were housed in cages under automatically controlled air-conditions of temperature (22±2°C), humidity (about 60%), and lighting (12:12-hr light-dark cycle). Mice were fed a commercial diet (Orient Bio) and tap water *ad libitum*.

Chonnam National University (CNU) Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the guidelines for animal experiments established by CNU.

Measurement of the swimming capacity An acrylic plastic pool (90×45×45 cm) was used to determine swimming capacity. The details were previously described (10). The mice were determined to be exhausted when they failed to rise to the surface of the water to breathe within a 7 sec period. A period of longer than 7 sec frequently resulted in drowning, while a period of less than 5 sec reduced the reproducibility of the test (10,11). The index of endurance swimming capacity was measured by the total swimming period until the exhaustion. The mice were administered FST (1 g/kg/day) via a stomach tube for 7 days. Saline was provided to the control group.

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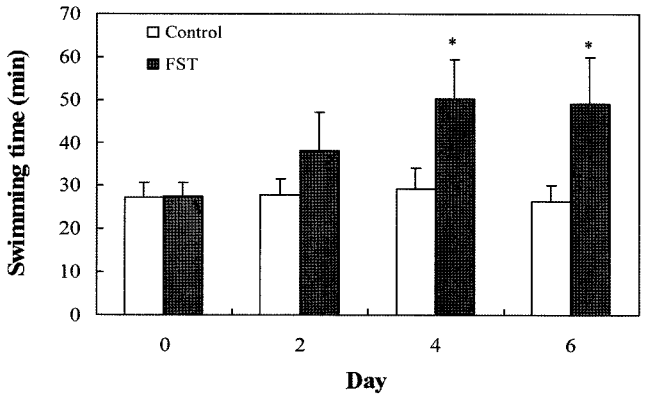

Fig. 1. Effect of fermented *ssanghwatang* (FST) on swimming capacity. The swimming capacities of mice were measured at a flow rate of 8 L/min and analyzed by Student's *t*-test. Each bar represents mean±SE (n=10). *p<0.05.

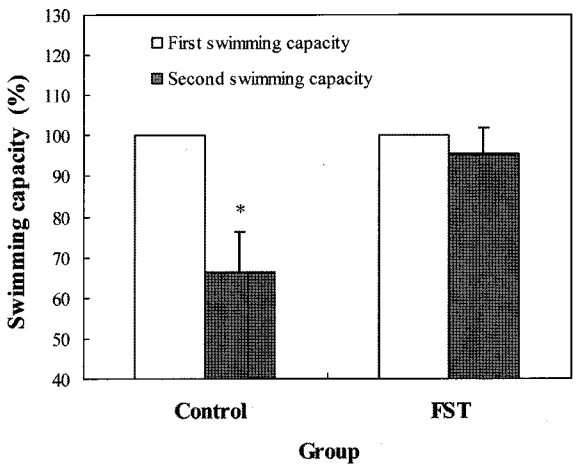

Fig. 2. Anti-fatigue effect of fermented *ssanghwatang* (FST) on consecutive swimming exercise of mice. Mice were administered FST or the control solution at 0.5 hr before the second swimming. The swimming capacities of mice were measured at a flow rate of 8 L/min and analyzed by Student's *t*-test. Each bar represents mean±SE (n=10). *p<0.05.

Determination of anti-fatigue effect The mice were forced to swim again after exhaustion was determined. The maximum swimming time during the second swimming, which followed the administration of FST (1 g/kg/day) and a rest period, was measured to evaluate the anti-fatigue effect on consecutive swimming exercise.

Analysis of serum lactic acid and glucose during swimming exercise Blood was taken from the tail at 15-60 min intervals during and after swimming. Serum lactate (Boehringer Mannheim Gmbh, Mannheim, Germany) and glucose concentrations (Wako Pure Chemical, Osaka, Japan) were analyzed with commercial kits.

Statistical analysis Values are presented as mean± standard error (SE). The significance of the mean difference between the control group and each treatment group was determined by Student's *t*-test. The level of $p<0.05$ was used as the criterion of statistical significance.

Results and Discussion

Enhancing effect of swimming capacity During the preliminary period, swimming time was determined 3 times. The mice were then separated into 2 groups (control and FST), each with similar mean swimming time. The FST group was administered FST (1 g/kg/day), followed by the measurement of the swimming capacity of each group at a current strength of 8 L/min every 2 days for a week. As shown in Fig. 1, the swimming capacity of the FST group was significantly enhanced over the control group at the beginning on the 4th day (29.27±4.73 vs. 50.27±9.05 min, $p<0.05$).

Anti-fatigue effect All mice were forced to swim until exhaustion was determined, followed by a 30 min rest period. The time to reach exhaustion was then determined during the second swim. Mice were administered FST at 30 min before the second swimming. The results suggest that FST prevented the physical fatigue caused by further exercise after the mice had been exhausted by an initial exercise (Fig. 2). It is supposed that FST enhanced the swimming capacity by diminishing fatigue in mice. The effect of FST on the recovery from exhaustion might be related to the resistance to physical intensive exercise. Shin *et al.* (12) reported that *gamisipjundaebotang* had anti-fatigue activity in mice in forced swimming tests. Also, Moriura *et al.* (13) observed anti-fatigue effect of *Agkistrodon blomhoffii* BOIE (a venomous snake, *mamushi* in Japanese) given orally (0.5 g/kg/day) for 3 successive days. It increased the swimming capacity of rats and prolonged the swimming time.

Continuous exercise causes fatigue, which can be reduced by the administration of nutritional regimens by suppressing fatigue relevance factors (14,15). Lactate is a key indicator of fatigue. Intensive exercise can increase lactate production to a point that exceeds the rate of lactate removal, which results in fatigue (16). The concentration of lactate in the blood was determined during and after forced swimming exercise. Serum lactate concentration of the control group gradually increased during swimming and reached a maximum value at the exhausted swimming time (Fig. 3). On the other hand, the FST group showed no increase in lactate level after 15 min of swimming. Though the lactate concentrations of both groups dropped during the rest period, the value of FST group was significantly lower than that of the control group ($p<0.01$). Wilber (17) indicated that intensive swimming to exhaustion resulted in a significantly elevated blood lactate level, and that the rate at which lactate accumulated in the blood showed an inverse relationship to swimming time. Therefore, it was suggested that FST made mice resistant to the physical fatigue by decreasing the lactate accumulation or production during the forced swimming exercise.

As shown in Fig. 3, the serum glucose concentration after 30 min of swimming in the FST group was similar to that in control group. However, the FST group exhibited a significantly higher glucose level after 60 min of limited swimming ($p<0.05$). The FST group showed a lower depletion of blood glucose and a reduced formation of blood lactate after swimming, which implies that the administration of FST reduced the ratio of carbohydrate

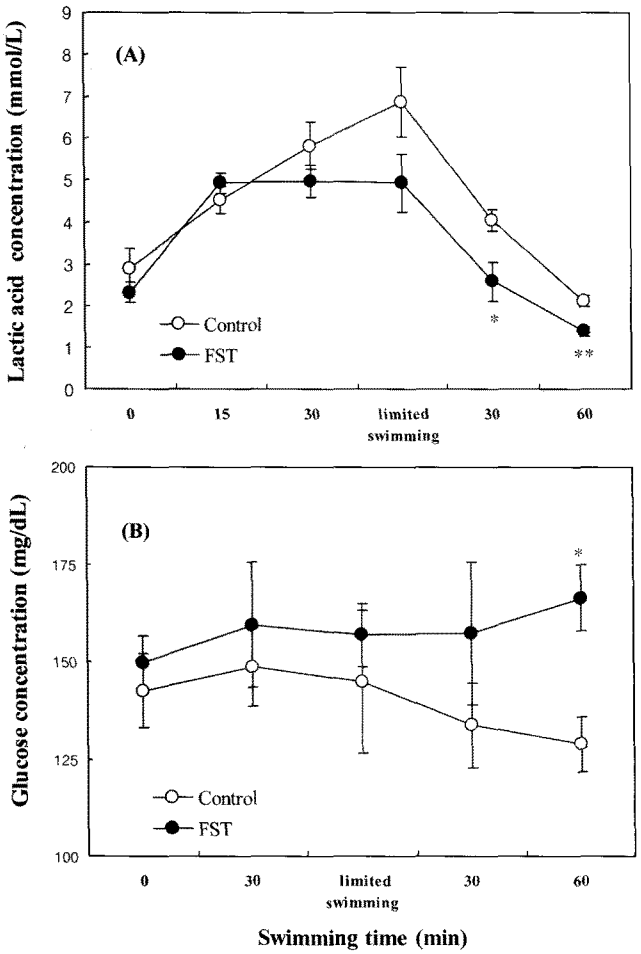

Fig. 3. Effect of fermented *ssanghwatang* (FST) on serum lactate (A) and glucose (B) level during swimming exercise. The swimming capacities of mice were measured at a flow rate of 8 L/min and analyzed by Student's *t*-test. Each bar represents mean±SE (n=10). *p<0.05, **p<0.01.

utilization during exercise, delaying the onset of fatigue.

Based upon these results, we conclude that the FST possessed the enhancing effects on swimming capacity and anti-fatigue by lowering blood lactate accumulation.

In preliminary experiment, we have analyzed the organic acid, vitamins, and amino acids of *ssanghwstang* and FST. The succinic acid, riboflavin, and thiamin of FST were higher than those of *ssanghwatang*. These finding suggest that the swimming capacity effect may be due to the activation of metabolism.

To evaluate the physiological and pharmaceutical effects of FST *in vivo*, more detailed understanding of the factors that enable the FST to exert anti-fatigue effect should be investigated. Therefore, further research on the characteristics of the FST will be required.

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