Abstract

Singi-Hwan (SH) is a widely used prescription for treating kidney inflammation in Korea. However, the protective effect of SH on maximal exercise performance (MEP) is not well studied. Fatigue is the physiological phenomenon after working and exercise to improve physical strength. The purpose of this study was to evaluate the MEP effect of SH using the FST model. To study the protective effect of SH on endurance maximal exercise performance by in vivo experiment. The mouse is largely divided into three groups; Non-swimming group, saline treatment - swimming load test group and SH processing swimming group. The swimming load test group was subdivided into the swimming control group (Control) and the Singi-Hwan supply group (SH). SH was orally administered 2 weeks before FST administration. After FST, immobility time, oxygen consumption was measured by physiological test, and serum was collected for biochemical analysis. Immobility, oxygen consumption and biochemical factors were increased FST - induced MEP. Immobility time was significantly decreased in SH treatment group compared to control group. Oxygen consumption was also significantly decreased by SH treatment. The increase of lactic acid and lactate dehydrogenase after FST was inhibited by SH treatment. Consumption of energy sources (free fatty acid and triglyceride) and energy recovery were improved by SH treatment after FST. In conclusion, SH suppressed the increase of immobility time, oxygen consumption and biochemical factors after FST. All the results suggest SH might be a potentially protective ingredient for the anti-fatigue functional food. In conclusion, SH can be used as a beneficial medium to improve maximal exercise performance and the ability to protect the body from fatigue. This study indicates that SH protects mice from physical fatigue and improves exercise performance. Therefore, it has a potential for the pharmacological effect of anti-fatigue. Our study provides new insight into the protective effects of SH on the fatigue status of mice. Additional studies are needed to find the mechanism of association between each single herb.

[Keywords] Protection, Singi-Hwan, Maximal Exercise Performance (MEP), Forced Swimming Test (FST), Lactic Acid

1. Introduction

Moderate exercise has an excellent effect in prevention of diseases and stress relieving, but excessive exercise causes body fatigue and fatigue[1][2][3][4][5]. Fatigue is a complex phenomenon, which means physical and mental exhaustion, in which normal muscle use is difficult and continuous exercise is difficult[6]. Control of fatigue is emerging as an important issue in modern society because chronic fatigue caused by fatigue can cause serious health problems[7].

Heavy work creates excess reactive oxygen species in the body, which can damage the muscles and organs of the body. Modern people, who are in a situation where it is difficult to avoid excessive work in the modern society where work and stress are high, are inevitably suffered from physical damage and fatigue. In order to improve this, the exercising ability or endurance enhancement supplements that were previously
only consumed by athletes became necessary for the modern public. As dietary supplements, they prefer to use nutritional supplements and health foods, but they are difficult to ingest because of their high price and difficulty in verifying efficacy. Even if diarrhea is bought and consumed, dietary supplements that are currently in use are reported to cause various side effects as steroid preparations, making it difficult to use them consistently[8]. Accordingly, a method of improving fatigue using herbal medicines and natural products has emerged as an alternative[9][10].

SH has been studied for various kidney diseases[11][12], and cerebral hemorrhage improvement effect and neuronal cell protection effect have been reported[13][14]. Various effects of SH are thought to be effective in improving exercise and endurance[12][13][14], but the effect of improving exercise endurance using SH has not been studied, and scientific proof is needed. In this study, in order to investigate the effects of SH on exercise endurance, forced swimming test model was used. And mouse immobility time, oxygen consumption in swimming, and biochemical indicators were analyzed to evaluate the endurance enhancement effect.

2. Methods

2.1. Preparation

All medicinal herbs of Singi-Hwan(SH) were purchased in the Omniherb(Yeongcheon, Korea) <Table 1>. SH(118 g) was extracted with 1 L distilled water for 150 min at 100℃, and then their residue was filtered through Whatman No.2 filter paper(Whatman Ltd., England). The extracts were concentrated using a rotary evaporator under vacuum condition, and the residual crude extracts were freeze-dried at -80℃. The SH extracts were stored at -20℃ during test. The yield was 26.84%.

Table 1. Composition of Shingi-Hwan.

<table>
<thead>
<tr>
<th>Name</th>
<th>Ratio(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rehmaniae rhizoma</td>
<td>28g</td>
</tr>
<tr>
<td>Dioscoreae rhizoma</td>
<td>16g</td>
</tr>
<tr>
<td>Corni fructus</td>
<td>16g</td>
</tr>
<tr>
<td>Schinsandrae fructus</td>
<td>16g</td>
</tr>
<tr>
<td>Poria</td>
<td>14g</td>
</tr>
<tr>
<td>Moutan radicis cortex</td>
<td>14g</td>
</tr>
<tr>
<td>Alismais rhizoma</td>
<td>14g</td>
</tr>
</tbody>
</table>

2.2. Animals

Male ICR mice(21-23 g) were purchased from Daehan Biolink(Eumseong, Korea) and housed individually in the home cages in a controlled room temperature(22±2℃) and humidity(50±5%) with a 12 h light-dark cycle (lights on at 7 a.m.), and allowed standard food pellets and tap water ad libitum. After acclimatization for 1 week, all experiments were carried out. In one experiment, a control group(a group swallowing water after water ingestion) and a control group(a swim group swallowing SH after ingestion) and a control group was used for the experiment. Water and SH(10 mg / kg) were orally administered to the experimental animals in the same amount for 2 weeks. A total of three independent experiments were performed. This protocol was approved by the Institutional Animal Care and Use Committee of Daegu Haany University(Approval number: DHU 2018-040).

2.3. Forced swimming test

In the forced swimming test, the control group that consumed water for 2 weeks, and
the experimental animals of the SH group were each subjected to a swimming adaptation training twice a week. Experimental animals consumed for 2 weeks were fed only with water for 16 hours before the forced swimming test and fasted for fast swimming test. Approximately 70% of water is added to the acrylic plastic water tank(70 × 70 × 60 cm) at a temperature of 24 °C to 26 °C, and the weight corresponding to 5% of the weight is suspended by the tail of the mouse according to method of Leichtweis et al.[15]. The burn-out was judged as if the mouse did not float to the surface for 7 seconds in water. For biochemical analysis, animals judged to be burned out were immediately sacrificed and blood was collected through the abdominal aorta. Serum was separated and stored at -70 °C.

2.4. Immobility measurement

After forced swimming, immobility was measured. Based on Abdul’s method[16], a typical immobility judgment was made such that the mouse only floated on the water, with only a small amount of movement in order to balance the body while only a part of the upper body including the face was exposed on the surface of the water. All experiments were done through blind test. When the upper body was submerged, it was judged that it was exhausted and it was excluded from the floating time.

2.5. Oxygen consumption measurement

Oxygen consumption was measured using an O₂/CO₂ analyzer(model RL-600, AlcoSystem Inc., Chiba, Japan) and a switching system (model ANI6-A-S, AlcoSystem Inc., Chiba, Japan).[17]

2.6. Biochemical serum analysis

The concentration of lactic acid, lactate dehydrogenase(LDH), triglyceride, TG, glucose and free fatty acid(FFA) in blood was measured using a commercial assay kit(Sigma, MO, USA) respectively.

2.7. Statistical analysis

All data are expressed as the means ± standard error of mean(SEM). The statistical significance of the differences between the groups were analyzed using one-way analysis of variance(ANOVA) followed by Newman-Keuls test in GraphPad Prism(version 5.03). Differences were considered statistically significant if p value less than 0.005 or 0.05.

3. Results

3.1. Effects of SH on forced swimming test (FST)

When fatigue is induced through forced swimming test, immobility time is greatly increased compared to normal mice. In contrast, immobility time was significantly decreased in the group treated with SH for 2 weeks(** P<0.05).

Figure 1. Effects of SH in the forced swimming test (FST).

Saline(−) or SH(10 mg/kg, SH) was administrated orally for 2 weeks. Then, mice were challenged with FST to examine immobility time. The similar results were obtained from three additional experiments(** P<0.05: significant as compared to FST alone.).

3.2. Effects of SH on oxygen consumption in the forced swimming test(FST)

If the body is damaged through forced swimming loads, the oxidative stress will be excessive and oxygen consumption will increase. Therefore, if oxygen consumption is
reduced, body damage is reduced and continuous exercise is possible. <Figure 2>, oxygen consumption was increased when forced swimming load was applied, while oxygen consumption was significantly decreased in SH-treated group(* P <0.005).

**Figure 2.** Effects of SH on oxygen consumption in the forced swimming test (FST).

Saline(-) or SH(10 mg/kg, SH) was administrated orally for 2 weeks. Then, mice were challenged with FST to examine oxygen consumption. The similar results were obtained from three additional experiments(*P<0.005: significant as compared to FST alone.).

### 3.4. Effects of SH on energy source in serum in the forced swimming test (FST)

In the early phase of exercise, carbohydrate is used, but in the latter half, fat is consumed by using fat such as free fatty acids, so fatty acid and lipid level in serum can be an index of maintenance of exercise endurance[18]. <Figure 4>, free fatty acid(FFA) and triglyceride levels in the mouse serum were significantly increased immediately after forced swimming, but the serum free fatty acid and triglyceride levels were significantly decreased in the SH-treated group. After 120 minutes of rest, the levels of free fatty acids and triglycerides were increased in the SH-treated group(* P <0.005, ** P <0.05). It shows that fatty acid consumption is reduced in the early part of the exercise and that SH in the latter part can help supply the energy source through fatty acids.

**Figure 4.** Effects of SH on serum energy factors in the forced swimming test.

Saline(-) or SH(10 mg/kg, SH) was administrated orally for 2 weeks. Then, mice were challenged with FST to examine free fatty acid(FFA).
and triglyceride. The similar results were obtained from three additional experiments(*$P<0.005$, **$P<0.05$; significant as compared to FST alone.).

4. Discussion

Fatigue caused by the body and damage can cause various disorders, which ultimately can damage the nervous system, endocrine system, immune system, and so on. Physical fatigue due to excessive work or exercise, and mental fatigue due to lack of sleep are also typical causes of poor quality of life[19][20]. In particular, physical fatigue leads to chronic fatigue if excessive accumulation of lactic acid occurs due to depletion of the energy source, so prevention and quick treatment are necessary. Lactic acid is produced to provide enough energy during high intensity exercise, regulating the pH of muscles and blood, and inducing various damages such as oxidative stress[21]. The forced swimming test model is a representative animal model of excessive bodily injury, similar to human phenomena such as depletion of energy source and increase in fatigue factor. As shown in <Figure 1>, the immobility time of mice is greatly increased due to loss of exercise capacity after forced swimming. In this study, the immobility time of the mice was greatly improved due to the administration of SH, which is a result of showing that SH can be involved in the improvement of the motor ability loss.

Recently, many studies on body injury or loss of athletic performance after excessive exercise have focused on oxidative stress. In fact, there are various reports that the degree of loss of athletic performance and the level of oxidative stress are related. It is also reported that antioxidant drugs may be involved in athletic performance enhancement[23], oxidative stress control may play a major role in athletic performance and endurance. Oxidative stress is caused by excessive oxygen consumption in the body, thereby increasing reactive oxygen species[22]. Since free radicals ultimately lead to DNA damage, excessive oxygen consumption can be thought of as ending with exercise endurance. In this study, we measured the oxygen consumption of the mice after forced swimming to investigate the effect of improving endurance. As shown in <Figure 2>, after the forced swimming, the oxygen consumption of the mouse was greatly increased and the endurance was decreased. However, oxygen consumption was decreased by SH administration, and it was determined that exercise endurance could be increased by this.

Muscle fatigue, muscle weakness, loss of athletic ability, and endurance decrease during the course of continuous use, due to depletion of ATP in muscle energy sources and accumulation of muscle fatigue[24]. Therefore, it is very important to remove fatigue substances in the muscles in order to improve endurance and maintain the ability of the muscles. In particular, lactic acid in the blood is a typical fatigue substance occurring during high-intensity exercise, and it is reported that rapid elimination is important for eliminating or alleviating fatigue[25]. In this study, the production of lactic acid and LDH was significantly increased during exercise, and lactate and LDH production were inhibited by SH administration <Figure 3>. It was concluded that SH could contribute to improvement of exercise endurance by eliminating fatigue element in muscle.

Energy source storage and supply is an important factor in exercise endurance. When exercising without an energy source, the physical fatigue increases, resulting in a significant reduction in endurance[26]. Typical sources of energy are fats and carbohydrates.
When the blast furnace energy source is limited, the fatigue improving agent should convert the neutral fat accumulated in the fat in the body into fatty acid to be introduced into the blood, thereby enabling energy generation. That is, the higher the levels of free fatty acids and triglycerides in the immediate post-exercise period, the depletion of the energy source becomes, and after the recovery time, the higher the serum level for recovery, the better the fatigue improvement. In this study, the levels of free fatty acids and triglycerides in the serum were decreased immediately after forced swimming of the SH, which showed that the SH was able to perform forced swim with low energy consumption. After 120 min of recovery time, the levels of free fatty acids and triglycerides in the serum were higher than those in the forced swimming group <Figure 4>. Therefore, it can be concluded that SH has a beneficial function in energy source storage and supply, which can contribute to the improvement of exercise endurance.

We could not determine the endurance improvement effect of SH with about 50 mice, but based on the results, we obtained the possibility of endurance improvement effect. It is believed that there is a great significance in suggesting the possibility of further analysis of related indicators from various organs of mouse for more accurate effect analysis.

5. Conclusion

The effect of the new kidney was observed in the mouse exercise endurance model using the forced swimming load, and the following conclusions were obtained. In the forced swimming model, the kidneys decreased the immobility time, oxygen consumption and fatigue factor of mice. In addition, the energy consumption of the new energy decreased and the recovery increased. These results suggest that in forced swimming model, the kidneys have the effect of improving the endurance of exercise, which can be applied to the development of drugs for fatigue improvement and muscle damage in the future. Further studies on the mechanism or mechanism of renal cirrhosis may be needed in the future. This study indicates that SH protects mice from physical fatigue and improves exercise performance.

6. References

6.1. Journal articles


Lead Author
Park Ju-sik / Keimyung University Professor
B.A. Keimyung University
M.A. Keimyung University
Ph.D. Keimyung University

Research field

Major career
- 2005~2014. Gyeongju University, Professor
- 2015~present. Keimyung University, Professor

Corresponding Author
Park Jin-han / Daegu Haany University Professor
B.A. Wonkwang University
M.A. Wonkwang University
Ph.D. Wonkwang University

Research field

Major career
- 2005~2014. Gyeongju University, Professor
- 2015~present. Daegu Haany University, Professor