J. Microbiol. Biotechnol. (2001), **11**(5), 902–905

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Swimming Endurance Capacity of Mice after Administration of Exo-Polymer Produced from Submerged Mycelial Culture of *Ganoderma lucidum*

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Received: June 6, 2001
Accepted: August 11, 2001

Abstract The effect of exo-polymer from *Ganoderma lucidum* on the swimming endurance capacity of mice was investigated. The administration of the exo-polymer (100 mg/kg body weight) increased the swimming endurance capacity of mice by about 10 min and reduced the muscle and liver glycogen exhaustion by 18.5% and 67.2%, respectively. A substantial decrease in serum lactate accumulation (50.7%) was also achieved under the influence of the exo-polymer. The exo-polymer was determined to be a glycoprotein with a molecular weight of 780 kDa and found to contain 82.8% carbohydrate and 17.2% protein.

Key words: Exo-polymer, *Ganoderma lucidum*, submerged mycelial culture, swimming endurance capacity

Recently, considerable attention has been focused on the search for natural compounds to enhance endurance. Yet, there are still very few reports available on this topic. In 1972, Cureton [3] demonstrated the endurance enhancing capability of wheat germ oil and identified the active ingredient as octacosanol. Costill *et al.* [2] reported on the effect of caffeine ingestion on metabolism and exercise performance. Much work has also been done on the endurance enhancing effect of capsaicin, a pungent component of red pepper [9, 10]. Kim *et al.* [12] found an increase in the swimming endurance capacity of mice under the influence of capsaicin. The current authors are also actively engaged in exploring new natural compounds with endurance enhancing capability, and as part of this exploration, *G. lucidum*, a mushroom of immense pharmaceutical importance [1, 7, 8, 15], was subjected to study. Preliminary experiments with the fruit-body and mycelia of *G. lucidum* did not yield any positive response in this regard. However, the exo-polymer produced from a submerged mycelial culture of *G. lucidum* exhibited some encouraging results by enhancing the swimming endurance capacity of mice and the data obtained is presented in the present communication.

To produce the exo-polymer, a submerged mycelial culture of *G. lucidum* (KCTC 0179BP) was carried out in 500-ml flasks containing 200 ml of a synthetic medium [16] on a rotary shaker (pH 4.5, 30°C, 120 rpm, 18 days). The recovery process [11] of the exo-polymer from the culture broth is shown in Fig. 1.

The exo-polymer obtained from the culture broth was dissolved in 0.2 M NaCl and subjected to gel filtration.

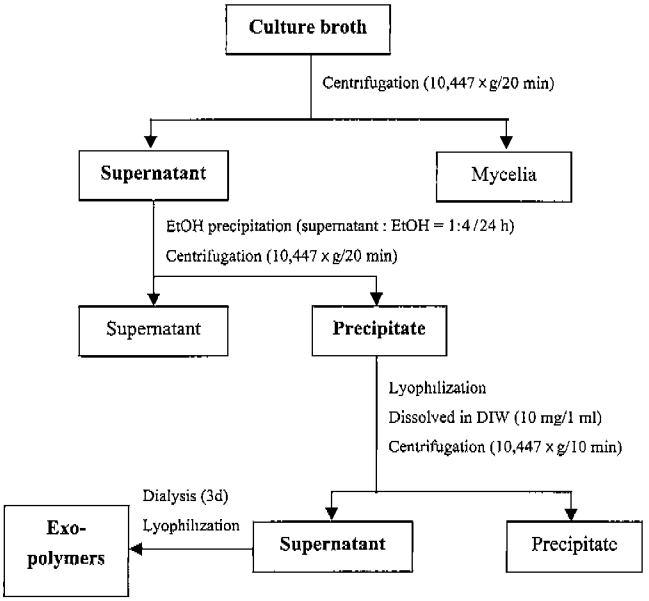

Fig. 1. Recovery process of exo-polymer from submerged mycelial culture of *Ganoderma lucidum*.

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This was performed in a column (2.6×99 cm) of Sepharose CL-6B, equilibrated with 0.2 M NaCl with a flow rate of 5 ml/tube volume. The molecular weight (MW) markers used were dextran (MW 2,000, 500, 70, 40, 10 kDa) and glucose (MW 180 Da) from Sigma Chemicals, U.S.A.

Male Std ddY 5-wk-old mice (Japan SLC, Hamamatsu, Japan) were used in the experiment and were housed in standard cages (33×23×12 cm, 4 mice per cage) under controlled conditions of temperature (22±0.5°C), humidity (50±5%), and a 12-h cycle of light and dark. During the experimental period, the mice were given free access to water and a commercial diet. The care and treatment of the experimental animals were carried out according to Kyoto University guidelines [14] for the ethical treatment of the laboratory animals.

An adjustable current water pool was used for measuring of the swimming endurance capacity. The details were previously described by Matsumoto *et al.* [14]. After a one-week preliminary period, during which the animals became accustomed to swimming, *i.e.*, 30 min swimming at a flow rate of 8 l/min a day, the mice were divided into two groups *i.e.*, control group [saline administered normal mice (n=12)] and GL group [*G. lucidum* exo-polymer administered normal mice (n=12)]. The exo-polymer (100 mg/kg body weight) or saline was administered orally *via* a stomach tube [17]. Thereafter, the mice were subjected to a swimming endurance test. After finishing the first round of swimming, the mice were given rest (recovery period) for 48 h before administration of another dose of the exo-polymer. This treatment cycle was continued for 3 weeks. The maximum swimming time of the mice was measured for each cycle of the experiment. To avoid circadian variations in the physical activity, the experiments were carried out between 10:00 and 18:00, during which a minimal variation in endurance capacity has been confirmed in mice. The animals were assessed to be fatigued when they failed to rise to the water surface to breathe within 7 seconds [14]. The swimming endurance capacity of the mice was measured using cross-experiments and the paired data were analyzed. To measure the endurance enhancing capacity in pre and post swimming phase in the control and GL groups, 48 h after finishing the 3 weeks of experimental period, half of the mice in the GL and control groups (post-swimming experimental group) were again administered the exo-polymer (100 mg/kg body weight) and saline. The mice were then subjected to a 20-min swimming load after being given a 2-h rest. The muscle and liver glycogen and serum lactic acid were measured immediately after the 20-min swimming load, and the values were compared with those of the other half of the 3-weeks experimental mice (stabilized group).

The glycogen concentrations of the gastrocnemius muscles were measured using an enzymatic technique, as described previously [6]. The serum L-lactic acid was measured by an enzymatic technique using a Kyowa Medes commercial kit (Determiner LA, Tokyo, Japan).

The total protein content of the exo-polymer was determined by the method of Lowry [13] using bovine serum albumin as the standard. The total carbohydrate content was determined by the phenol sulfuric acid method [4] using a glucose and galactose mixture (1:1) as the standard. The amino acid and carbohydrate were measured by HPLC and gas chromatographies based on the hydrolysis and acetylation methods, as described previously [16].

The data were expressed as means±SE. The swimming capacity of each group was analyzed using a paired Duncan's multiple-range test [5]. A level of $p<0.05$ was used as the criterion for statistical significance.

Food Intake and Body Weight

The changes in the food intake and body weight during the 3-week period are presented in Table 1. The administration of exo-polymer could not significantly affect either the body weight or food intake. Moreover, oral administration of the exo-polymer caused no changes in gross behavior and none of the animals died. Therefore, it can be stated that there were no harmful effects in the mice following oral administration of the exo-polymer of *G. lucidum*.

Swimming Endurance Capacity

The swimming endurance of the GL and control group of mice was measured after each experimental cycle. The time it took for the mice to become fatigued after each swimming test during the 3-week experimental period is presented in Fig. 2. The effect of the exo-polymer on the swimming capacity was not so prominent up to 10 days of the swimming experiment. However, after 12 days, the GL group showed a significant improvement in the swimming time and could swim 10 min more than the control group.

Table 1. Change in body weight and food intake.

Day	Body weight (g/3wk)		Food intake (g/day)	
	Control*	GL**	Control*	GL**
0	34.50±0.49	34.49±0.47	-	-
2	35.23±0.63	35.63±0.63	4.64±0.06	5.31±0.06
4	35.80±0.59	36.23±0.57	4.45±0.05	4.98±0.05
6	35.49±0.61	35.96±0.69	4.56±0.06	4.79±0.06
8	35.15±0.59	35.74±0.54	3.91±0.05	4.37±0.05
10	35.13±0.61	35.71±0.49	4.12±0.06	4.69±0.04
12	35.53±0.57	35.85±0.53	4.09±0.05	4.48±0.05
14	36.18±0.59	36.92±0.48	4.22±0.05	4.48±0.04
16	36.37±0.53	36.96±0.47	4.01±0.05	4.22±0.04
18	36.64±0.59	37.07±0.42	3.81±0.05	4.09±0.04
20	36.78±0.53	37.15±0.56	3.65±0.05	4.06±0.05

Values are means±SE (n=12).
*Control: saline-administered normal mice; **GL: exo-polymer of *G. lucidum*-administered normal mice.

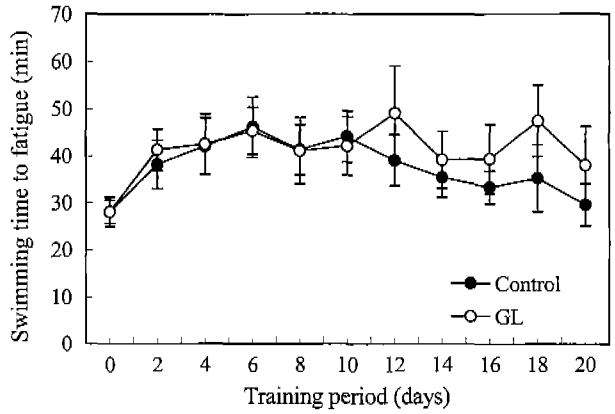

Fig. 2. Swimming time to fatigue during chronic swimming training in a current pool.
Values are means±SE (n=12).

Concentration of Serum Lactic Acid, Muscle Glycogen, and Liver Glycogen

In order to determine the possible reason for the enhanced endurance, the content of the muscle and liver glycogen and serum lactic acid of the mice in the post-swimming experimental and stabilized groups were measured (Fig. 3).

At the end of the 3-week experiment (stabilized group), the control group registered relatively higher values for the muscle and liver glycogen and serum lactic acid levels compared with those of the GL group, although not significant. A significant drop in the muscle and liver glycogen in both the GL and control groups was noticed after the 20-min swimming test (post-swimming experimental group). However, the GL group conserved significantly more glycogen than the control group. The control group retained about 34.42% of the muscle glycogen, whereas 43.11% was conserved in the GL group. As much as 85.29% and 95.30% of the liver glycogen was retained in the control and GL groups, respectively. A significant increase in lactic acid accumulation was registered in the control group of mice after the 20-min swimming test, where an 18.61% enhanced level of lactic acid was recorded. In contrast, the accumulation of lactic acid in the GL group was insignificant. Generally, in a fatigue state, the lactic acid in serum accumulates and glycogen in liver and muscle is exhausted [2].

From the present investigation, it is evident that the *G. lucidum* exo-polymer may have an endurance enhancing capability. It was also found that the exo-polymer did not have any effect on the swimming time during the first 10 days of the swimming test, and only enhanced the swimming capacity after 12 days. Accordingly, it would seems that the experimental mice needed a 'period of habituation' to respond to the exo-polymer treatment. The liver and muscle glycogen levels in the experimental mice, measured 48 h after the 3-week swimming endurance test (stabilized group), did not show any increased accumulation.

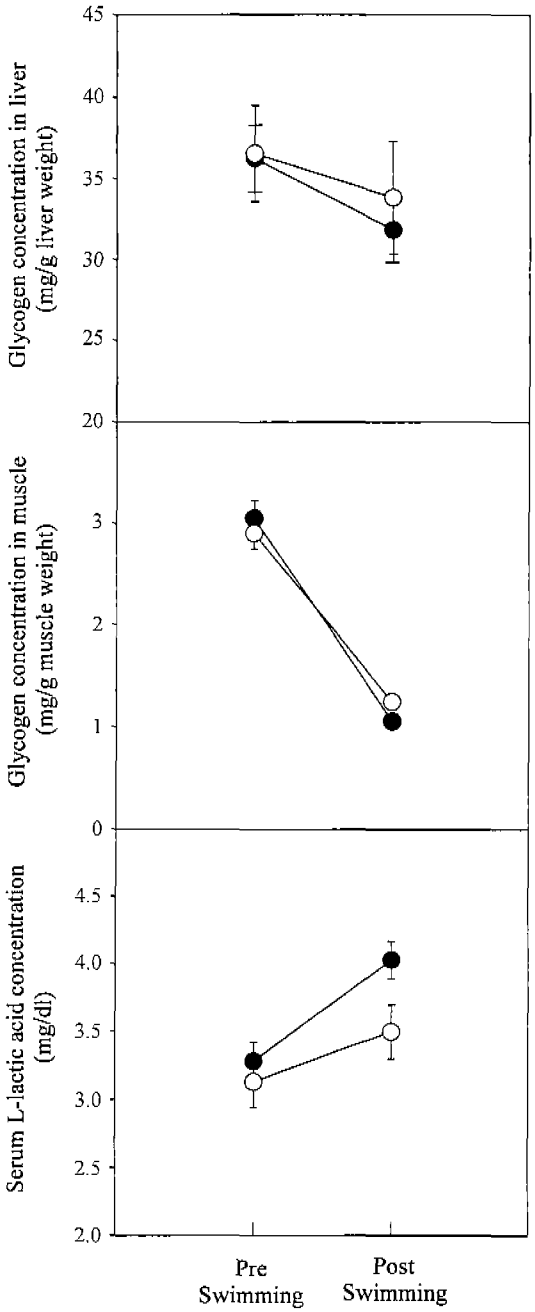

Fig. 3. Concentration of muscle glycogen, liver glycogen, and serum L-lactic acid of stabilized and 20-min swimming groups.
Values are means±SE (n=6). ●, Control group; ○, GL group.

However, higher liver and muscle glycogen conservations and lower lactic acid accumulation, indications of better endurance, were observed in the exo-polymer-treated mice when measured after a 20-min swimming load (post-swimming experimental group). Therefore, it is likely that the effect of the exo-polymer was transient and only effective in conservation of reserved glycogen. No long-term effect was observed in the accumulation of glycogen in

the experimental animals. The reason for a 'period of habituation' is still unclear, however, since this was only a preliminary study, further work is needed to evaluate the energy metabolism resulting in enhanced endurance after treatment with the exo-polymer.

Chemical Analysis of Exo-Polymer

The exo-polymer obtained from the mycelial culture broth yielded only one peak, when passed through the Sepharose CL-6B column, with a MW of 780 kDa.

The exo-polymer was found to contain 82.8% carbohydrate and 17.2% protein, and no acidic sugar was detected. Six different kinds of sugar constituted the carbohydrate moiety. Histidine, serine, alanine, and valine were detected as the major amino acids of the protein part [16]. It would appear that the exo-polymer of *G. lucidum* studied in the present investigation is a glycoprotein. However, whether the carbohydrate or protein part is responsible for the endurance enhancing activity is still unclear. Further study is currently in progress.

Acknowledgments

This research was supported by a Taegu University research grant, 2000. We would also like to thank Professor Tohru Fushiki (Laboratory of Nutrition Chemistry, Kyoto University, Japan) for kindly providing laboratory facilities to perform this work.

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