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Antifatigue Effect of *Chlorella vulgaris* in Mice

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클로렐라의 항 피로 효과 연구

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Abstract

A unicellular algae, *Chlorella vulgaris* (CV), was used as a biological response modifier. The effect of CV on forced swimming test and blood biochemical parameters related to fatigue was investigated. Blood urea nitrogen (BUN); creatine kinase (CK); lactic dehydrogenase (LDH); glucose (Glc); total protein (TP); and albumin were determined. CV was orally administered to mice in the range of 0.05 to 0.15 g/kg/day. A forced swimming test results on 3 and 7 day after administration of CV, showed that immobility time was decreased in the CV-administered group (0.15 g/kg). In addition, the contents of BUN in the blood serum were decreased in CV-fed group. The contents of CK and LDH were tended to decrease, but not statistically significant. The plasma Glc level was increased in CV-fed groups (0.05 and 0.1 g/kg) compared to control group. It had no effect on the elevation of TP and albumin level. The results indicate that CV could improve physical stamina.

Key words : *Chlorella vulgaris*, forced swimming test, immobility time, blood parameters

Introduction

Chlorella vulgaris (CV) is a freshwater unicellular, microscopic algae, widely used as a food supplement in the forms of tablets, capsules, extract liquid or a food additive in Japan[1]. It claims health benefits including improvement of immune function[2] and improvement in control of hypertension, fibromyalgia and ulcerative colitis. Numerous human and animal experiments have documented various pharmacological effects of CV and CV extract, which includes improvement in hypertension[3] and lipid metabolism[4], enhancing anti-tumor[4] and

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antibacterial activities[5], and promotion of dioxin excretion[6]. Kanouchi *et al.*[3] treated male Sprague-Dawley rats with CV powder orally to study its effects on serum antibody levels and antibody production in splenocytes and mesenteric lymphocytes. Their results confirmed that CV enhanced immunoglobulin (Ig) M and IgG antibody production by circulating splenocytes and mesenteric lymphocytes. Sarac *et al.* [7] investigated whether chlorella dietary supplements to relieve signs and symptoms, improve quality of life, and normalize body functions in people with chronic illnesses, specifically fibromyalgia, hypertension, and ulcerative colitis. Daily dietary supplementations with chlorella reduce high blood pressure, lower serum cholesterol levels, accelerate wound healing, and enhance immune functions. Halperin *et al.*[8] reported that chlorella-derived dietary supplement did not have any effect in increasing the antibody response to influenza vaccine in the overall study population, although there was an increase in antibody response among participants aged 50~55 years. Recipients of 400 mg of the chlorella who were 55 years of age or younger had significantly higher geometric mean antibody titres against influenza A/New Caledonia 21 days after vaccination and against B/Yamanashi 7 days after vaccination.

Forced swimming test (FST) is behavioral test for rodents, which predicts the efficacy of antidepressant treatments[9~11]. This test induces the development of immobility as a reflection of helplessness when subjected to an inescapable situation (tank of deep water). In this paradigm, mice are placed in the tank for an extended period. After an initial swimming period, the animal exhibits immobility behavior considered as a depression-like response. Also, FST is used to examine whether a certain agents has anti-fatigue effect[12~15] and used for endurance test[16,17].

The blood urea nitrogen (BUN), creatine kinase (CK), lactic dehydrogenase (LDH), glucose (Glc), total protein (TP), and albumin are blood biochemical parameters related to fatigue. The BUN test is a routine test used primarily to evaluate renal function. Urea is formed in the liver as the end product of protein-metabolism. During digestion, protein is broken down to amino acids. Amino acids contain nitrogen, which is removed as NH_4^+ (ammonium ion), while the rest of molecule used to produce energy or other substances needed by the cell[16]. Serum CK and LDH is known to be accurate indicators of muscle damage[18,19]. The normal function of CK in cells is to add a phosphate group to creatine, turning it into the high-energy molecule phosphocreatine. Phosphocreatine is burned as a quick source of energy by cells. Exercising muscles convert Glc to lactate. Lactate is released into the blood and is eventually taken up by the liver. The liver converts lactate back to Glc and releases Glc into the blood. This Glc is then taken up by resting muscles, red blood cells, and other tissues. Energy for exercise is derived initially from the breakdown of glycogen, and later from circulating Glc released by the liver and from non-esterified fatty acids[20]. As is commonly known, Glc level is decreased immediately after exercise. TP is a rough measure of serum protein, whereas albumin, the main protein in blood, is the key to the regulation of the osmotic pressure of blood. Protein measurements can reflect nutritional state, kidney disease, liver disease, and many other conditions. Enzymes, some hormones, hemoglobin, LDL, fibrinogen, immunoglobulins are some examples of proteins. The present study was aimed to examine the effect of CV on FST and the biochemical parameters related to the fatigue, such as serum BUN, CK, LDH, Glc, TP, and albumin.

Materials and Methods

1. Animals

Male ICR mice weighing 16(18 g (Orient Co., LTD, Sungnam, Gyeonggi-do, Republic of Korea) were used in these experiments. The mice were divided into four groups; 1) control group; 2) 0.05 g/kg CV treatment group; 3) 0.1 g/kg CV treatment group; and 4) 0.15 g/kg CV treatment group. Each groups was housed under following laboratory conditions: temperature 23±1℃, humidity 40~60%, 12:12-L/D cycle, lights on at 07:00 h. Food and water were available *ad libitum*. All the experiments were carried out between 10:00 and 15:00 h in testing rooms adjacent to the animal rooms. Mice were treated in accordance with the current law and the NIH Guide for Care and Use of Laboratory Animals.

2. Treatment of CV

The CV used in this study was supplied by Daesang Corp. WellLife (Seoul, Republic of Korea). It was

dissolved in distilled water and used at 0.05, 0.1, and 0.15 g/kg dose. The extract or distilled water was administered orally for 1 week.

3. FST

During the 6 min of the FST, the duration of immobility was measured as previously described by Porsolt *et al.*[9]. The apparatus consisted of two Plexiglas cylinders (height: 25 cm, diameter: 10 cm) placed side by side in a Makrolon cage filled with water (10 cm height) at 23~25 ℃. Two mice were tested simultaneously for a 6 min period inside vertical Plexiglas cylinders; a nontransparent screen was placed between the two cylinders to prevent mice from seeing each other. After a delay of 2 min, the total duration of immobility was measured during a period of 4 min. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. After the first measurement of immobility time, the mice were divided into a control group and three concentrations of CV groups (0.05, 0.1, and 0.15 g/kg) to match the swimming time in each group. The second measurement of immobility was performed 2 days after distilled water or CV treatment.

4. Preparation and Ingredient Analysis of Blood Serum

In order to clarify its mechanisms, we assessed the levels of several blood biochemical parameters in mice after FST. At the end of the experiment, mice were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg). After anesthetization, blood was withdrawn from heart of forced swimming-treated mice into syringes. Then, serum was prepared by centrifugation at 10,000 rpm at 4℃ for 10 min. Contents of BUN, CK, LDH, Glc, TP, and albumin were determined by the autoanalyzer (Hitachi 747, Hitachi, Japan).

5. Statistical Analysis

Results were expressed as the mean±S.E. of independent experiments, and statistical analyses were performed by one-way analysis of variance (ANOVA) with Tukey, and Duncan post hoc test to express the difference among the groups. All statistical analyses were performed using SPSS v12.0 statistical analysis software. A value of $P<0.05$ was considered to indicate statistical significance.

Results

1. Effect of CV on Immobility

When mice were placed into the cylinders for the first time, they swam vigorously around apparently searching for an exit. The immobility time was decreased in the CV-treated groups (0.1 and 0.15 g/kg) in comparison with the distilled water-treated group ($P<0.05$; Fig. 1. 2a). After 4 days, the immobility time was decreased in the CV-

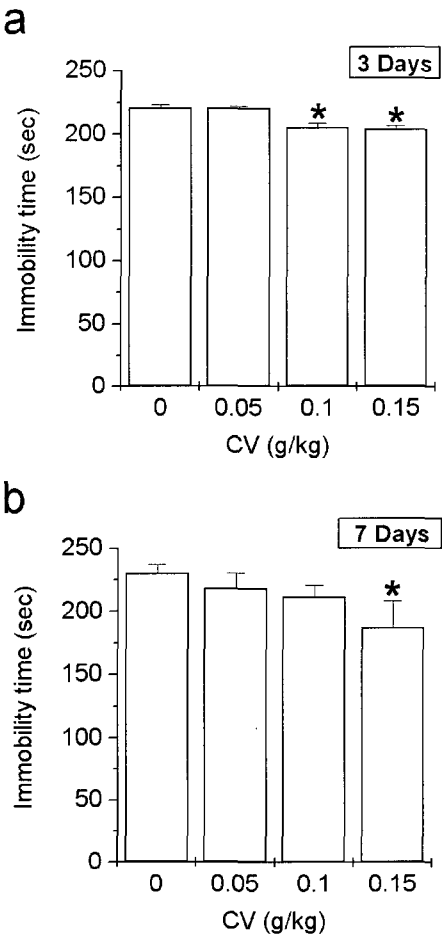

Fig. 1. Effect of CV on forced swimming-induced immobility in mice. One day after the 1st measurement of immobility, the administration of CV was started; this continued for a total of 7 days. (a) On the 2nd day after the first administration. (b) On the 7th day after the first administration.
Each data value indicates the mean±S.E.
* Significantly different from distilled water-treated group at $P<0.05$.

treated groups in comparison with the distilled water-treated group ($P<0.05$; Fig. 1. 2b). Especially, in the 0.15 g/kg of CV treated-group (187 ± 21.7 s), the immobility times were significantly decreased in comparison with the distilled water-treated group (230 ± 7.0 s).

2. Effects of CV on Renal Function

The BUN level in 0.05 and 0.1 g/kg treated-group was decreased significantly compared with it in distilled water-treated group (Fig. 2).

3. Effect of CV on Muscle Damage

As shown in Table 1, when CV (0.05~0.15 g/kg, for 7 days) was administered orally to mice, the CK and LDH levels showed a tendency to decrease; however, this decrease was not significant.

4. Effect of CV on Energy Source

Finally the effect of CV on energy source was investigated by checking the Glc, TP, and albumin levels in serum. The Glc level was significantly increased by CV administration (0.05 and 0.1 g/kg; $P<0.05$; Fig. 3). However, the TP and albumin levels were not affected by CV (Table 2).

Discussion

Biological effects of CV on tumors, bacteria, and mitogenes have been described previously[3,5,6]. Most of these

Table 1. The ingredients of CV

(mg/100g)		(mg/100g)	
Glutaminc acid	5061	Calcium	1005.2
Lysine	4460.3	Cystine	734
Leucine	4041.4	Potassium	686.9
Aspartic acid	3935.7	Magnesium	481.3
Alanine	3690.4	Folic acid	116.7
Arginine	2946.9	β-carotene	37.6
Glycine	2494.2	Sodium	30.2
Valine	2464	Niacin	20.67
Chlorophyl	2325.2	Fiber, total dietary	20
Tyrosine	2100.7	Vitamin D	8.8
Proline	2094.4	Zinc	6.5
Threonine	2084	Pantothenic acid	2.1
Phenylalanine	2002.6	Pheophorbide	0.2
Serine	1875.7	Copper	0.1
Histidine	1807.6	Chrome	0.05
Tryptophan	1643.9	Iodine (μg/100g)	99.9
Isoleucine	1531.9	Vitamin K (μg/100g)	26
Methionine	1201.4	Vitamin D (μg/100g)	8.8
Phosphorus	1028.1		

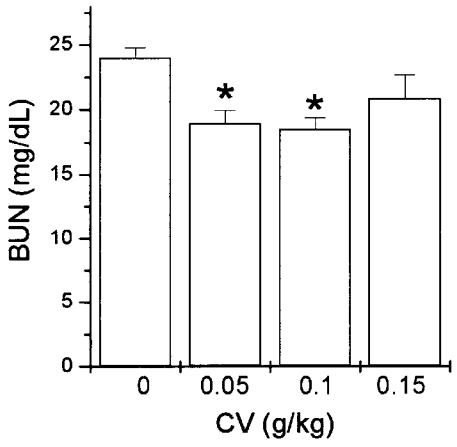

Fig. 2. Effect of CV on BUN levels in mice.
Each data value indicates the mean±S.E.
* Significantly different from distilled water-treated group at $P<0.05$.

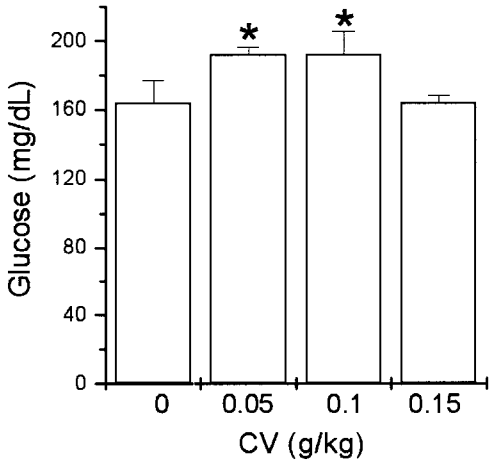

Fig. 3. Effect of CV on Glc levels in mice.
Each data value indicates the mean±S.E.
* Significantly different from distilled water-treated group at $P<0.05$.

Table 2. Effect of CV on LDH and CK in mouse serum[a]

CV (g/kg)	LDH (IU/L)	CK (IU/L)
0	678.0±57.2	549.2±62.1
0.05	520.2±31.0	429.6±39.2
0.1	580.5±67.5	499.6±20.1
0.15	542.6±46.7	466.2±55.2

[a] CV (p.o. for 7 days) was administered orally to mice. Each data value indicates the mean±S.E.

Table 3. Effect of CV on TP and albumin in mouse serum[a]

CV (g/kg)	TP (mg/dL)	albumin (g/dL)
0	5.1±0.2	3.1±0.1
0.05	5.3±0.1	3.1±0.0
0.1	5.4±0.1	3.0±0.1
0.15	5.3±0.2	3.2±0.1

[a] CV (p.o. for 7 days) was administered orally to mice. Each data value indicates the mean±S.E.

studies provide strong evidence for the hypothesis that CV administration, particularly in immunocompromised hosts, may enhance immunity. The results of the current study show that the duration of immobility was shortened by the administration of CV. These phenomena suggest that the decreased duration of immobility in mice may be caused by a change of certain metabolites in the system. The dosage of CV was determined 0.05～0.15 g/kg as stated other researches[6,21~23]. To confirm the suitability of dosage, we also assessed plasma alanine aminotransferase and aspartate aminotransferase activities to evaluate hepatic dysfunction. In view of the result achieved, 0.05～0.15 g/kg CV treatment did not affect liver injury.

In general, the swimming exercise is known to induce blood biochemical changes[24]. The BUN levels of rugby players during a summer training camp significantly increased[25]. The BUN level tended to decrease by the administration of CV for 7days. In particular by 0.05 and 0.1 g/kg CV treatment, the BUN level was decreased significantly (Fig. 2). Because the BUN test is a routine test used primarily to evaluate renal function[26], this result means that CV can be applied to the improvement of renal function after exercise. The CK and LDH are known to be accurate indicators of muscle damage[16,17] and catalyze the interconversion of pyruvate and lactate. Therefore, the CK and LDH levels increase immediately after exercise. Our results showed that glucose level tended to decrease by the administration of CV (0.05～0.15 g/kg), but did not statistically significant (Table 1). These data suggest that CV may prevent muscle damage. The glucose level decreased immediately after exercise. Glucose level was increased by the administration of CV (0.05 and 0.1 g/kg; Fig. 3). On the other hand the TP and albumin levels showed no change as compared with those of the distilled water-treated mice.

In general, swimming exercise is known to induce blood biochemical changes[27]. Although the statistical difference was weak, tendencies among some changes were found in biochemical parameters after application with CV. This change appears to be related to the decrease of immobility time. As such, our results indicate that immune-enhancing metabolisms of mice were influenced by CV administration. However, further studies to clarify the detailed mechanisms involved in the immune-enhancing properties of CV are necessary to support the present findings.

In conclusion, CV treatment decreased the immobility time during FST and changed the metabolites related to physical state. Therefore, the present results suggest the possibility that CV may be useful for the development of physical strength.

요 약

단세포 조류 생물인 *Chlorella vulgaris* (클로렐라)는 다양한 생리 활성을 가진 기능성 소재로 이용되고 있다. 본 연구에서는 강제 수영 부하 실험과 혈액 생화학적 지표에 대한 클로렐라의 효과에 대해 연구하였다. 혈액 생화학적 지표로는 Blood urea nitrogen (BUN), creatine kinase (CK), lactic dehydrogenase (LDH), glucose (Glc), total protein (TP), albumin을 혈액 생화학적 지표로 측정하였다. 매일 0.05, 0.1, 0.15 g/kg 농도의 클로렐라를 각각 실험 군별로 마우스에 구강 투여했다. 클로렐라를 투여한지 3일, 7일째 되는 날 강제 수영 부하 실험을 시행한 결과 0.15 g/kg 클로렐라를 투여한 그룹에서 유의적으로 부동시간을 감소시켰다. 또한 혈청 중 BUN 수치를 낮췄으며, CK, LDH 수치는

감소하는 경향을 나타냈다. 클로렐라 투여 시 혈 중 Glc 수치는 높아졌으나, TP와 albumin 수치는 변화가 없었다. 이상의 결과들은 클로렐라가 육체적 지구력 향상 효과가 있는 것을 시사하고 있다.

색인어 : 클로렐라, 강제 수영 부하 실험, 부동 시간, 혈중 생화학적 지표

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