Effect of Conclevan on Endurance Capacity in Mice

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The purpose of this study was to clarify the anti-fatigue effect of Conclevan, which is mainly composed of liver hydrolysate, *via* a forced swimming test using mice. Conclevan was administered to mice for 6 weeks, and a forced swimming test was conducted to measure swimming time. After six weeks, the blood ammonia and glutamine concentrations were measured. In the Conclevan administration group, swimming time increased significantly compared to the swimming control group. In the swimming control group, an increase in blood ammonia and a decrease in blood glutamine were observed, relative to the non-swimming control group. In the Conclevan administration group, the increased blood ammonia and decreased blood glutamine induced by swimming were significantly reduced, compared to the swimming control group. The mRNA expression levels of the hepatic enzymes of the urea cycle (carbamoyl-phosphate synthetase, argininosuccinate synthetase, and arginase) and glutamine synthesis (glutamate dehydrogenase and glutamine synthetase) were significantly increased in the Conclevan administration group, compared to the swimming control group. The results of this study demonstrated the anti-fatigue effects of Conclevan. This product may inhibit an increase in the fatigue-inducing ammonia concentration in the blood by increasing the expression of hepatic enzymes, which convert ammonia to urea, leading to increased swimming time. In addition, Conclevan may prolong swimming time by increasing the hepatic synthesis of glutamine, which is an important amino acid for supplying energy in muscles.

Key words liver hydrolysate; anti-fatigue effect; ammonia; glutamine

Fatigue is a common symptom found in both sick and healthy people, and chronic or accumulated fatigue can severely affect an individual's performance. In addition, long-term accumulated fatigue can lead to death as a result of overwork.[1,2] Recently, it has been reported that various natural substances are effective in preventing or reducing fatigue. For example, prolonging exercise duration can be achieved by ingesting capsaicin, which increases blood catecholamine concentration[3,4]; green tea extract, which promotes fat burning in skeletal muscles[5,6]; and red mold rice, which reduces the oxidative stress induced by exercise.[7]

Conclevan has been commonly used as a pharmaceutical agent in Japan. Conclevan contains liver hydrolysate, which is obtained *via* enzymatic degradation of liver, as a main ingredient, along with heart extract and vitamins (Table 1). Conclevan is used for nutritional fortification, improvement of weak constitutions, and nutritional support in cases of fatigue, declining physical strength after an illness, nutritional disorders, and gastrointestinal disorders. It is believed that liver hydrolysate consists primarily of amino acids[8] and exerts an anti-fatigue effect by enhancing liver functions[9—11]; however, there is little scientific evidence to support this claim. The purpose of this study is to clarify the anti-fatigue effect of Conclevan in an animal study and to study the mechanism of its effects.

Table 1. Conclevan Components and Content in a 30 mL Daily Dose

Component	Content
Liver hydrolysate	600 mg
Heart extract	300 mg
Thiamine disulfide (vitamin B_1)	13.5 mg
Riboflavin (vitamin B_2)	3 mg
Pyridoxine hydrochloride (vitamin B_6)	3 mg
Cyanocobalamin (vitamin B_{12})	30 μg

The forced swimming test is a widely-used experimental method to evaluate anti-fatigue treatments.[12,13] The effects of Conclevan in mice were examined using this test, and the mechanism underlying the anti-fatigue effect of Conclevan was investigated, with ammonia, a fatigue-inducing substance, as the main focus.[14—16]

MATERIALS AND METHODS

Materials Conclevan was donated by Nissui Pharmaceutical Co., Ltd. (Ibaraki, Japan). TRI Reagent was purchased from Sigma-Aldrich Corp. (St. Louis, MO, U.S.A.). Primers were purchased from Invitrogen Corp. (Tokyo, Japan). A high capacity cDNA synthesis kit was purchased from Applied Biosystems (Foster City, CA, U.S.A.), and iQ SYBR green supermix was purchased from Bio-Rad Laboratries (Hercules, CA, U.S.A.). All other reagents were of the highest commercially-available grade.

Animals and Treatments Four-week-old male ddY mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). Mice were kept at 24±1°C and 55±10% humidity, with 12 h of light (artificial illumination; 08:00—20:00). After one week of pre-feeding, the swimming times of all mice were measured. Based on the measured swimming times, the mice were divided into 5 groups: 1) non-swimming control group, 2) swimming control group, 3) swimming sucrose group, 4) swimming Conclevan 1.5% group, and 5) swimming Conclevan 3.0% group. The mice were then fed *ad libitum* with a commercial rodent diet (MF, Oriental Yeast, Tokyo, Japan) and were given purified water (Groups 1 and 2), 0.7% sucrose (Group 3), 1.5% Conclevan (Group 4), or 3.0% Conclevan (Group 5), respectively, by water bottle, for six weeks. During this period, the forced swimming test was conducted with mice in Groups 2,

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Table 2. Primer Sequences

Target	Accession number	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
Carbamoyl-phosphate synthetase	NM_001080809	GGTGATGATCGGGGAGAG	GGGTCAGCATCTCTCAGTC
Argininosuccinate synthetase	NM_007494	GGTGCAGGTGTCTGTCTTC	ATAGTCGCCCTGCACGTTC
Arginase	NM_007482	GCTGGACCCAGCATTCAC	CCTGAAAGGAGCCCTGTC
Glutamate dehydrogenase	NM_008133	TGGAGTCTGGCAGGCTTC	AGCCTGCTGCTGGTGAAG
Glutamine synthetase	NM_008131	GGATGCAGTACCGGGAAAG	GGCTACCTGTCCAACATCTG
18S rRNA	X00686	GTCTGTGATGCCCTTAGATG	AGCTTATGACCCGCACTTAC

3, 4, and 5 to measure swimming times. The mice were fasted for 18 h before the forced swimming test, and were given only the test substances *ad libitum*. The amount of food and water intake was measured once per week. Blood samples were collected under ether anaesthesia after measurement of the swimming time at the sixth week. Afterwards, the liver was removed, instantly frozen in liquid nitrogen, and stored at −80°C.

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animal, as adopted by the Committee on Animal Research at Hoshi University.

Measurement of Swimming Time The forced swimming time was measured using the method described by Matsumoto *et al.*[13] An acrylic pool (90×45×45 cm) filled with water to a depth of 38 cm was used. A current was generated in the pool by a pump and maintained at a flow rate of 8 L/min. The temperature of the water was maintained at 25±0.5°C with a water heater and thermostat. The mice were made to swim until the end point of the swimming test, which was defined as the time point when the mice could not resist the current and failed to rise to the surface of the water to breathe within 7 s. In this study, the mice were subjected to a 10 min preliminary swim before 10 min of rest. After the rest, the mice were made to swim until the end point of the swimming test, which was recorded as the swimming time.

Blood Analysis Blood samples were centrifuged (1000×*g* for 15 min at 4°C), and the serum was stored at −80°C until use. Serum glucose levels were quantified using a Glucose CII Test (Wako Pure Chemical Industries, Tokyo, Japan). Serum corticosterone levels were quantified using a Corticosterone enzyme-linked immunosorbent assay (ELISA) kit (Assay Designs, Inc., Ann Arbor, MI, U.S.A.). Serum lactic acid levels were quantified using an F-kit D/L-lactic acid (Roche Diagnostic, Basel, Switzerland). Serum ammonia levels were quantified using an F-kit ammonia (Roche Diagnostics). Serum glutamine levels were quantified using an EnzyChrom™ Glutamine Assay Kit (BioAssay Systems, Hayward, CA, U.S.A.).

RNA Preparation from Liver RNA was extracted from about 15 mg of frozen liver using TRI Reagent. The resulting solution was diluted 50-fold using Tris/ethylenediaminetetraacetic acid (EDTA) buffer (TE buffer), and the purity and concentration (μg/mL) of RNA were calculated by measuring absorbance at 260 and 280 nm using a U-2800 spectrophotometer (Hitachi High Technologies, Tokyo, Japan).

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) A high-capacity cDNA synthesis kit was used to synthesise cDNA from 1 μg of RNA. TE buffer was used to dilute the cDNA 20-fold to prepare the cDNA TE buffer solution. The expression of target genes was detected using the prepared primers listed in Table 2 for real-time RT-PCR. To each well of a 96-well PCR plate, 25 μL of iQ SYBR green supermix, 3 μL of forward primer of the target gene (5 pmol/μL), 3 μL of reverse primer (5 pmol/μL), 4 μL of cDNA TE buffer solution, and 15 μL of RNase-free water were added. For the expression analysis of 18S ribosomal RNA (rRNA), 4 μL of a cDNA TE buffer solution was prepared by diluting 20-fold from the above-mentioned solution using TE buffer. The denaturation temperature was set at 95°C for 15 s, annealing temperature at 56°C for 30 s, and elongation temperature at 72°C for 30 s. The fluorescence intensity of the amplification process was monitored using the My iQ™ single color real-time RT-PCR detection system (Bio-Rad Laboratories), and the mRNA expressions were normalised to the 18S rRNA.

Statistical Analysis Numerical data are expressed as means±standard deviation. Tukey's test for multiple comparisons was used to identify statistical differences between groups; results with $p<0.05$ were considered significant.

RESULTS

Body Weight, Food Intake, and Water Intake After the end of administration, the body weight of the non-swimming control group (normal group) was approximately 42 g. The body weight of the swimming control group, swimming sucrose group, swimming 1.5% Conclevan group, and swimming 3.0% Conclevan group were all similar to that of the non-swimming control group (Fig. 1A).

The food intake of the non-swimming control group was approximately 5 g/mouse/d. The food intake of the swimming control group, swimming sucrose group, swimming 1.5% Conclevan group, and swimming 3.0% Conclevan group were all similar to that of the non-swimming control group (Fig. 1B).

The water intake of the non-swimming control group was approximately 5 mL/mouse/d. The water intake of the swimming control group, swimming sucrose group, swimming 1.5% Conclevan group, and swimming 3.0% Conclevan group were all similar to that of the non-swimming control group (Fig. 1C).

Effect of Conclevan on Swimming Time Little change was observed in the swimming times of the swimming sucrose group from the second to sixth week of administration, and no difference in swimming time was observed between the swimming sucrose group and swimming control group. However, in the swimming 1.5% Conclevan group, a significant increase in swimming time was observed after the fifth week, compared to the swimming control group and swim-

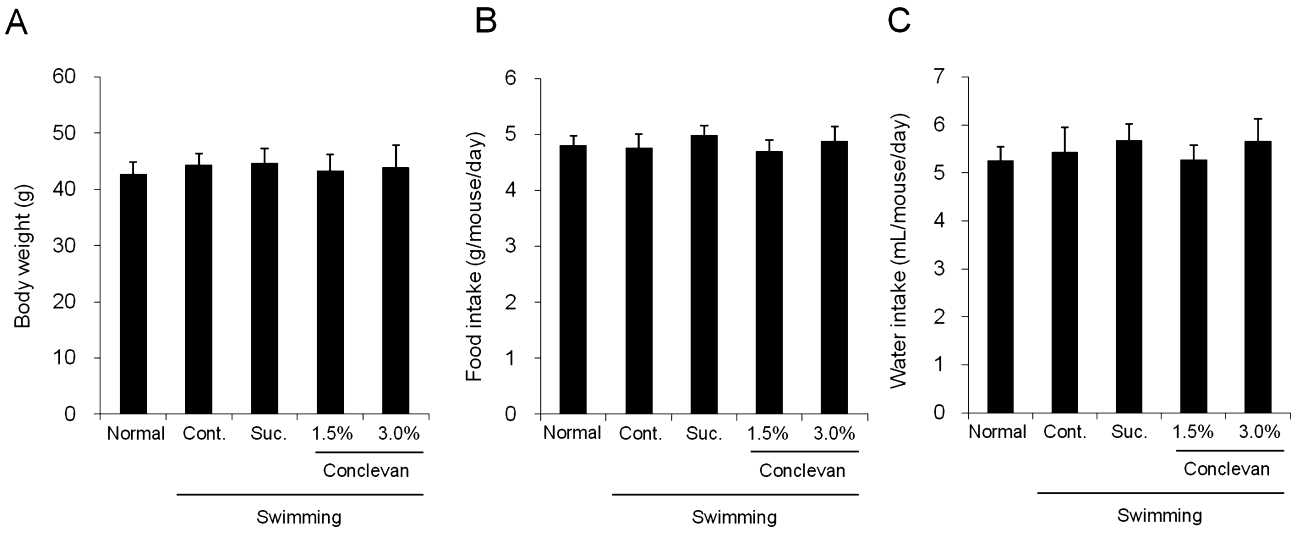

Fig. 1. Body Weight and Food and Water Intake

Mice were given purified water, 0.7% sucrose, a 1.5% aqueous solution of Conclevan, or a 3.0% aqueous solution of Conclevan for six weeks. The forced swimming test was conducted once per week from the second week to the sixth week during the six week period of the study. The non-swimming control group (normal group) was given purified water for six weeks. After six weeks, body weight was measured (A). Food intake (B) and water intake (C) were measured once per week during the six-week period. Data represents the mean±S.D. of 10 mice per group.

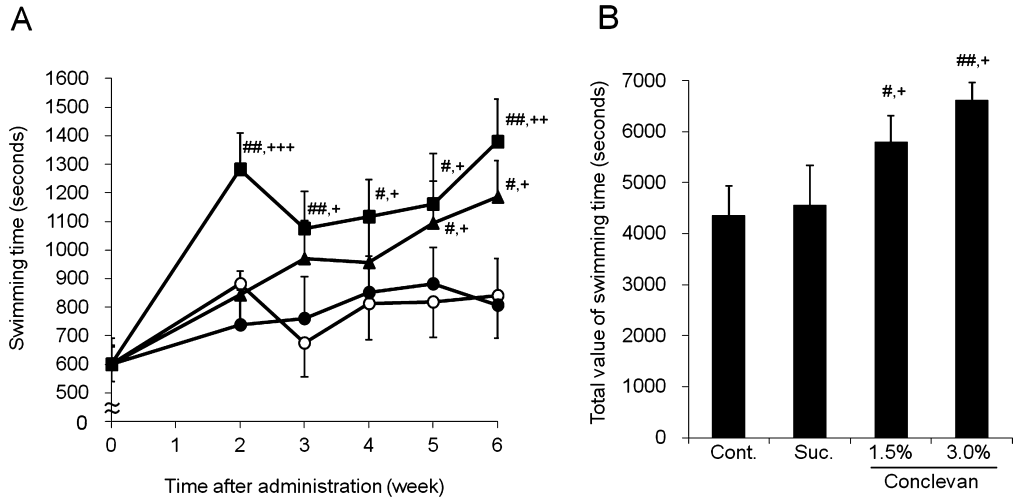

Fig. 2. Effect of Conclevan on Swimming Time

Mice were given purified water (○), 0.7% sucrose (●), a 1.5% aqueous solution of Conclevan (▲), or a 3.0% aqueous solution of Conclevan (■) for six weeks. The forced swimming test was conducted once per week from the second week to the sixth week during a six week period to measure the swimming time (A). The total value of each six swimming times was calculated (B). Data represents the mean±S.D. of 10 mice per group. Tukey's test: $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* swimming control group; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ *vs.* swimming sucrose group.

ming sucrose group. In the swimming 3.0% Conclevan group, a significant increase in swimming time was observed at all points of measurement, compared to the swimming control group and swimming sucrose group (Fig. 2A).

The total value of each of the six swimming times was approximately 4200 s in both the swimming control group and swimming sucrose group, while the total value was approximately 6000 and 6500 s in the swimming Conclevan 1.5% and 3.0% groups, respectively, showing an increase in total swimming time with an increase in the amount of administration of Conclevan. A significant difference was observed in the total swimming time, especially in the swimming Conclevan 3.0% group, compared to the swimming control group and swimming sucrose group (Fig. 2B).

Effects of Conclevan on Serum Glucose, Corticosterone, Lactic Acid, Ammonia, and Glutamine Levels Each test substance was administered for six weeks, and blood samples were collected soon after the last forced swimming test to analyse the serum components (Fig. 3).

The blood glucose level in the non-swimming control group (normal group) was approximately 130 mg/dL. The blood glucose level in the swimming control group was approximately 60 mg/dL, which showed a significant decrease, due to swimming. The blood glucose level in the swimming sucrose group was significantly higher than that in the swimming control group. The blood glucose levels in both the swimming Conclevan 1.5% and 3.0% groups were significantly higher than that in the swimming control group. However, no signifi-

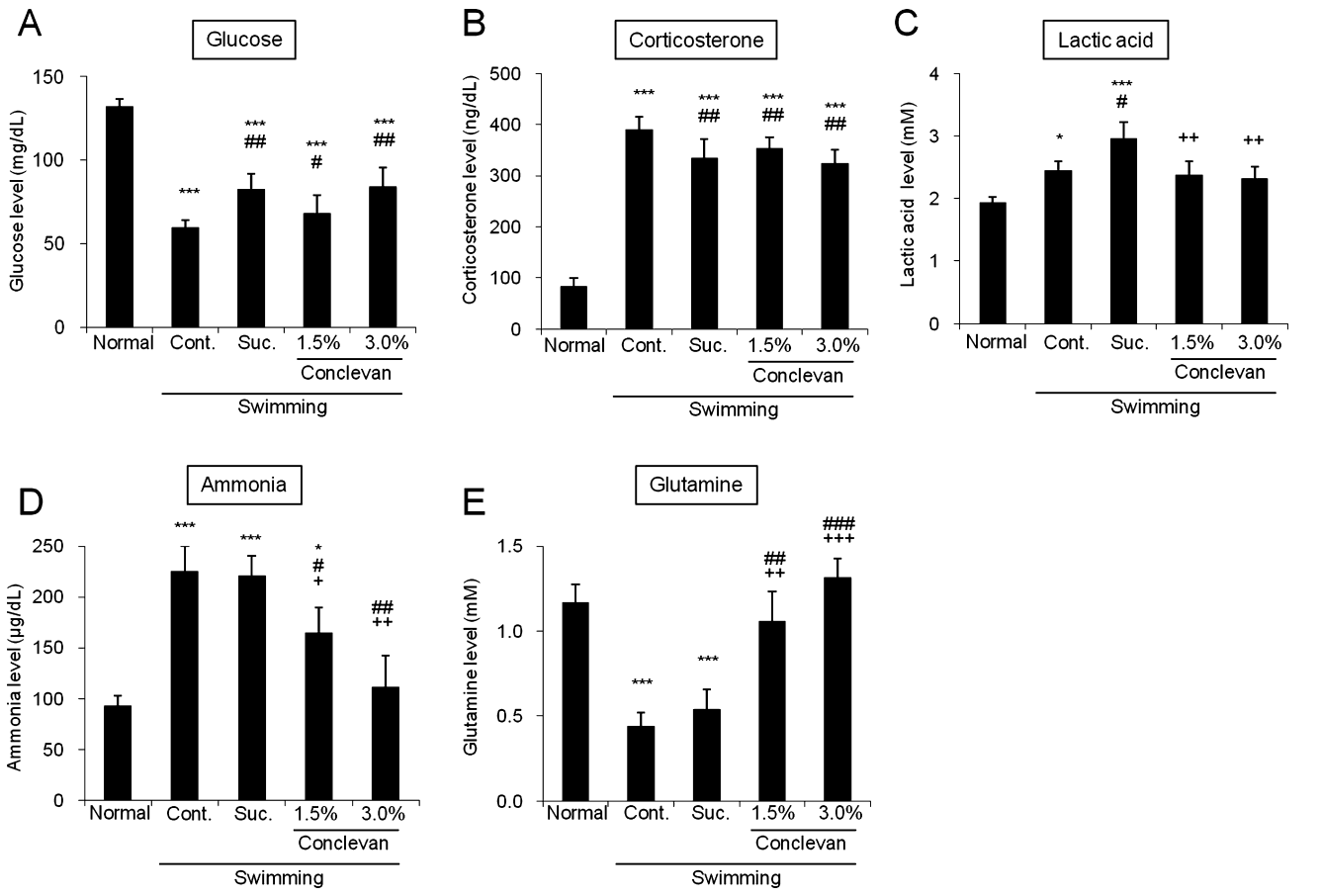

Fig. 3. The Effect of Conclevan on Blood Components

Mice were given purified water, 0.7% sucrose, a 1.5% aqueous solution of Conclevan, or a 3.0% aqueous solution of Conclevan for six weeks. The forced swimming test was conducted once per week from the second week to the sixth week during a six week period. Soon after the measurement of the swimming time at the sixth week, a blood sample was collected, and the serum glucose level (A), corticosterone level (B), lactic acid level (C), ammonia level (D), and glutamine level (E) were measured. The non-swimming control group (normal group) was given purified water for six weeks. Data represents the mean±S.D. of 10 mice per group. Tukey's test: $^{*}p<0.05$, $^{***}p<0.001$ *vs.* non-swimming control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ *vs.* swimming control group; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ *vs.* swimming sucrose group.

cant difference was observed in blood glucose level between the swimming sucrose group and Conclevan administration groups (Fig. 3A).

The blood corticosterone level in the non-swimming control group was approximately 100 ng/dL. The blood corticosterone level in the swimming control group was approximately 400 ng/dL, which showed a significant increase, due to swimming. The blood corticosterone levels in the swimming sucrose group and the swimming Conclevan 1.5% and 3.0% groups were all significantly lower than that in the swimming control group (Fig. 3B).

The blood lactic acid level in the non-swimming control group was approximately 2.0 mM. The blood lactic acid level in the swimming control group was approximately 2.5 mM, which showed a significant increase, due to swimming. The blood lactic acid level in the swimming sucrose group was significantly higher than that in the swimming control group. The blood lactic acid levels in the swimming Conclevan 1.5% and 3.0% groups were not significantly different from that in the swimming control group. However, the blood lactic acid levels in the Conclevan administration groups were significantly lower than that in the sucrose group (Fig. 3C).

The blood ammonia level in the non-swimming control group was approximately 100 μg/dL. The blood ammonia level in the swimming control group was approximately 220 μg/mL, which showed a significant increase, due to swimming. The blood ammonia level in the swimming sucrose group was very similar to that in the swimming control group, while the blood ammonia levels in the swimming Conclevan 1.5% and 3.0% groups were significantly lower than that in the swimming control group (Fig. 3D).

The blood glutamine level in the non-swimming control group was approximately 1.2 mM. The blood glutamine level in the swimming control group was approximately 0.4 mM, which showed a significant decrease, due to swimming. No significant difference was observed between the blood glutamine levels of the swimming sucrose group and the swimming control group. The blood glutamine levels in the swimming Conclevan 1.5% and 3.0% groups increased significantly to 2-fold and 2.5-fold the level found in the swimming control group, respectively (Fig. 3E).

mRNA Expression Levels of the Enzymes of Urea Cycle in the Liver Figure 4 shows the mRNA expression levels of carbamoyl-phosphate synthetase, argininosuccinate synthetase, and arginase, which are important enzymes in the urea cycle in the liver.

The mRNA expression level of carbamoyl-phosphate synthetase increased approximately 2-fold with swimming. The mRNA expression level of carbamoyl-phosphate synthetase in the swimming sucrose group was very similar to that in

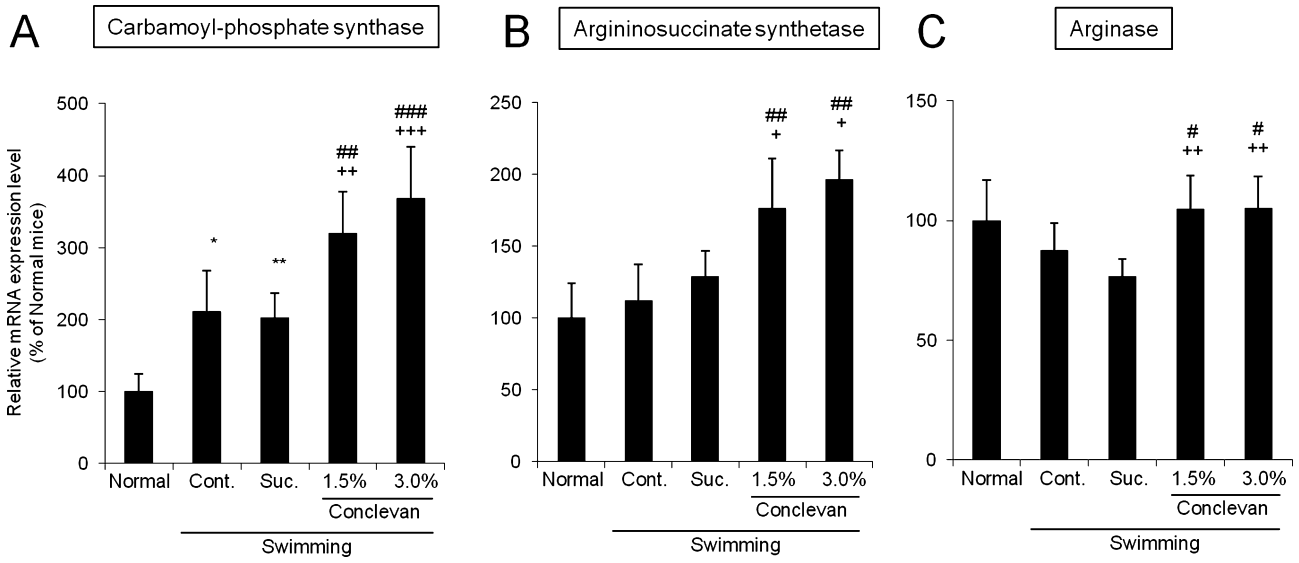

Fig. 4. mRNA Expression Levels of Enzymes of the Urea Cycle in the Liver

Mice were given purified water, 0.7% sucrose, a 1.5% aqueous solution of Conclevan, or a 3.0% aqueous solution of Conclevan for six weeks. The forced swimming test was conducted once per week from the second week to the sixth week during a six week period. Soon after the measurement of the swimming time at the sixth week, the liver was removed, and the mRNA expression levels of carbamoyl-phosphate synthase (A), argininosuccinate synthetase (B), and arginase (C) was measured by real-time RT-PCR. 18S rRNA was used as a house-keeping gene, and the averages of values derived from the non-swimming control group (normal group) were expressed as 100%. The non-swimming control was given purified water for six weeks. Data represents the mean±S.D. of 10 mice per group. Tukey's test: $^{*}p<0.05$, $^{**}p<0.01$ *vs.* non-swimming control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ *vs.* swimming control group; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ *vs.* swimming sucrose group.

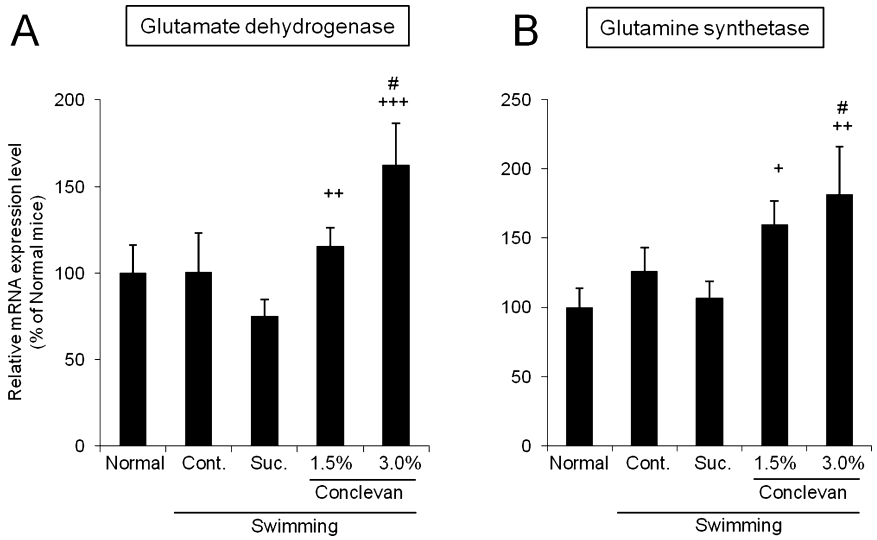

Fig. 5. mRNA Expression Levels of Enzymes Involved in Glutamine Synthesis in the Liver

Mice were given purified water, 0.7% sucrose, a 1.5% aqueous solution of Conclevan, or a 3.0% aqueous solution of Conclevan for six weeks. The forced swimming test was conducted once per week from the second week to the sixth week. Soon after the measurement of the swimming time at the sixth week, the liver was removed, and mRNA expression levels of glutamate dehydrogenase (A) and glutamine synthetase (B) was measured by real-time RT-PCR. 18S rRNA was used as a house-keeping gene, and the averages of values derived from the non-swimming control group (normal group) were expressed as 100%. The non-swimming control was given purified water for six weeks. Data represents the mean±S.D. of 10 mice per group. Tukey's test: $^{\#}p<0.05$ *vs.* swimming control group; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ *vs.* swimming sucrose group.

the swimming control group. The mRNA expression levels of carbamoyl-phosphate synthetase in the swimming Conclevan 1.5% and 3.0% groups increased significantly, compared to the swimming control and swimming sucrose groups (Fig. 4A).

The mRNA expression level of argininosuccinate synthetase showed no change with swimming. The mRNA expression level of argininosuccinate synthetase in the swimming sucrose group was very similar to that in the non-swimming control and swimming control groups. The mRNA expression levels of argininosuccinate synthetase in the swimming Conclevan 1.5% and 3.0% groups increased significantly, compared to the swimming control and swimming sucrose groups (Fig. 4B).

The mRNA expression level of arginase showed a decrease with swimming. The mRNA expression level of arginase in the swimming sucrose group was similar to that in the swimming control group. The mRNA expression levels of arginase in the swimming Conclevan 1.5% and 3.0% groups increased significantly, compared to the swimming control and swimming sucrose groups (Fig. 4C).

mRNA Expression Levels of the Enzymes of Glutamine Synthesis in the Liver Figure 5 shows the expression levels of mRNA of glutamate dehydrogenase and glutamine synthetase, which are important enzymes in glutamine synthesis pathway in the liver.

The mRNA expression level of glutamate dehydrogenase showed no change with swimming. The mRNA expression level of glutamate dehydrogenase in the swimming sucrose group was very similar to that in the non-swimming control and swimming control groups. The mRNA expression levels of glutamate dehydrogenase in the swimming Conclevan 1.5% and 3.0% groups increased significantly, compared to the swimming control and swimming sucrose groups (Fig. 5A).

A relatively minor change in the mRNA expression level of glutamate synthetase was observed with swimming. The mRNA expression level of glutamate synthetase in the swimming sucrose group was similar to that in the non-swimming control and swimming control groups. The mRNA expression levels of glutamate synthetase in the swimming Conclevan 1.5% and 3.0% groups increased significantly, compared to the swimming control and swimming sucrose groups (Fig. 5B).

DISCUSSION

Conclevan is a pharmaceutical agent that is used for nutritional fortification and the improvement of weak constitutions in Japan. In this study, Conclevan was administered to mice at doses 5-fold (Conclevan 1.5% administration group) and 10-fold (Conclevan 3.0% administration group) higher than the clinical dose, after which a forced swimming test was conducted to evaluate the effectiveness of Conclevan. The hypothesised mechanism of action was also evaluated, focusing on the fatigue-inducing substance, ammonia.[14—16)]

In order to investigate the caloric intake and the intake of the test substances, the amounts of food and water consumed were measured. The results showed that the intake amount of food in each group was nearly constant throughout the duration of administration (Fig. 1A), indicating that the calorie intake from food was equal in all groups. In addition, there were no differences between any groups in the amounts of water consumed (Fig. 1B). The results indicated that the Conclevan intake in the mice in the Conclevan 3.0% administration group was 2-fold that in the Conclevan 1.5% administration group, and that the calorie intakes in the Conclevan 3.0% administration group and the sucrose group were comparable. The reason for establishing the sucrose group, whose calorie intake is comparable to the Conclevan 3.0% administration group in this study, is that it is known that the swimming time in forced swimming test is affected by calorie intake.

There was little difference in swimming time between the swimming sucrose and the swimming control groups throughout the test period, whereas the swimming time in the Conclevan 3.0% administration group increased significantly, compared to the swimming control group at all points of measurement (Fig. 2). These results suggested that the increase in the swimming time in the Conclevan 3.0% administration group is not attributable to the increase in caloric intake.

What caused the increase in the swimming time in the Conclevan administration group? The concentrations of serum glucose, corticosterone, lactic acid, ammonia, and glutamine are known to serve as indicators of accumulated fatigue and stress caused by exercise.[12,17—19)] The analysis of these components in the blood samples collected after the last measurement of the swimming time at the sixth week of the administration showed decreases in the serum concentrations of glucose and glutamine, along with increases in the serum concentrations of corticosterone, lactic acid, and ammonia in the swimming control group compared to the non-swimming control group (Fig. 3). These results showed that the forced swimming was associated with an accumulation of fatigue and stress markers. In addition, the observation that the results of this study agree with previous reports suggests that the forced swimming test was an appropriate method for this study.[12,17—19)] In this forced swimming test, the sucrose group did not show a significant improvement in most of the components examined. However, in the Conclevan administration groups, significant reductions were observed in the decrease in the serum concentration of glutamine, as well as in the increases in the serum concentrations of ammonia (Fig. 3).

Vigorous exercise increases the production of ammonia, resulting in an increased level of ammonia in blood. This accumulated ammonia exerts a toxic effect on muscles and the brain, leading to decreased exercise capacity.[20)] Therefore, it is possible to enhance exercise capacity by decreasing the concentration of ammonia in the blood.[12,15)] In addition, vigorous exercise consumes not only glucose but also glutamine, which is a primary amino acid for supplying energy in muscles.[21)] Therefore, it is possible to enhance exercise capacity by increasing the concentration of glutamine in the blood. When ammonia accumulates in the body due to exercise, it is detoxified in the liver. The major route of this detoxification is the urea cycle, in which ammonia is converted to urea,[22)] but there is another route of detoxification, in which ammonia is converted to glutamine by glutamate dehydrogenase and glutamine synthetase.[23)] The former route takes place mainly in the cells surrounding the hepatic portal vein, while the latter route takes place mainly in the cells surrounding the hepatic vein. There was no difference in the activity of important urea cycle enzymes such as carbamoyl-phosphate synthetase, argininosuccinate synthetase, and arginase[22)] or glutamine synthesis enzymes such as glutamate dehydrogenase and glutamine synthetase[23)] between the swimming sucrose group and the swimming control group. However, in the Conclevan administration groups, the mRNA expression levels of all of the above-mentioned enzymes increased significantly (Figs. 4, 5). Based on these results, it is suggested that Conclevan possibly induced the expression of important enzymes that convert ammonia to urea in the liver, resulting in an inhibition of the increase in ammonia concentration in the blood, eventually leading to an inhibition of muscle fatigue and a prolongation of swimming time. In addition, the prolongation of the swimming time by Conclevan administration may be attributable to the increase in glutamine synthesis in the liver.

The effect of Conclevan on the blood concentration of ammonia and glutamine as well as on various hepatic enzymes under normal conditions (*i.e.*, non-exercise) was examined. The results showed that there were no changes in the blood concentrations of ammonia and glutamine or in the mRNA expression levels of various hepatic enzymes in a non-swimming 3.0% Conclevan group, compared to the non-swimming control group (data not shown). Therefore, it is likely that Conclevan exerts an anti-fatigue effect by increasing the activ-

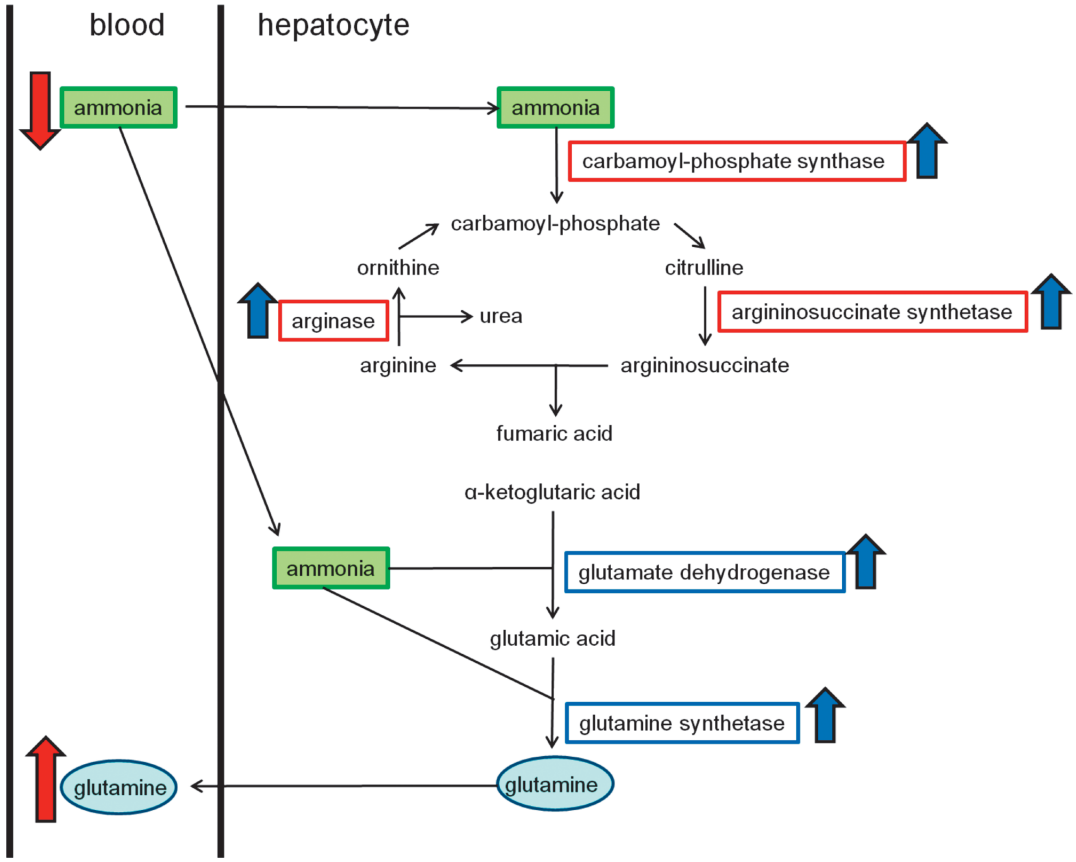

Fig. 6. Suggested Mechanism for the Anti-fatigue Effect of Conclevan

ity of hepatic enzymes involved in ammonia metabolism when the ammonia level is increased by exercise, an effect which results in a decrease in the concentration of ammonia.

Conclevan contains various vitamins, such as vitamin B_1, vitamin B_2, vitamin B_6, and vitamin B_{12} (Table 1). Nozaki *et al.* have reported that the anti-fatigue effect of vitamin B_1, which is contained the most much vitamin in Conclevan, was not significant when vitamin B_1 was orally administered to rats at 50 mg/kg.[24] In addition, it was reported that the anti-fatigue effect of vitamin B_1 was not significant when vitamin B_1 was orally administered to rats at 70 mg/kg.[25] In our study, vitamin B_1 was administered to mice at a dose of 1.5 mg/kg (Conclevan 3.0% administration group). Therefore, it is not considered that vitamin B_1 is the active component of anti-fatigue effect.

This study clarified the anti-fatigue effect of Conclevan in an animal test. Moreover, we succeeded in identifying the mechanism of action contributing to this effect, as shown in Fig. 6.

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