Effects of *Eleutherococcus senticosus* on the oxidative enzyme activity in mouse skeletal muscle

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Abstract

Aqueous extract from the root of *Eleutherococcus senticosus* MAXIM, Araliaceae (AERES), a Chinese traditional medicine, has been used to improve physical health. In the present paper, we examined the effect of long and short term administrations of AERES on oxidative enzyme activities in skeletal muscles of mice.

In the experiment of the long term administration, ICR mice were given AERES *p.o.* at 170 mg/kg per day (6 days/week) for 9 weeks starting at 5 weeks of age. They were sacrificed at 8, 10, 12, 14 and 19 weeks of age. The succinate dehydrogenase (SDH) activity in the gastrocnemius muscle of the mice treated with AERES increased significantly at 8, 12 and 14 weeks of age, compared with that of the age-matched control mice. The SDH activity remained at a higher level even at 19 weeks of age (5 weeks after the final administration of AERES). The malate dehydrogenase (MDH) activity increased only at 12 weeks of age.

In the experiment of the short term administration, AERES was given to ddY mice *p.o.* at 170 mg/kg per day for 10 consecutive days starting at 13 weeks of age. The mice were sacrificed on the day after the final treatment. The SDH activity increased significantly in gastrocnemius and tibialis anterior muscles of the mice treated with AERES, and the MDH activity tended to increase.

These results suggested that AERES enhances aerobic metabolism through increase of the SDH activity of skeletal muscles.

Key words *Eleutherococcus senticosus* MAXIM, Araliaceae, succinate dehydrogenase (SDH), malate dehydrogenase (MDH), skeletal muscle, mice.

Abbreviations ES, *Eleutherococcus senticosus* ; AERES, aqueous extract from root of *Eleutherococcus senticosus* ; MDH, malate dehydrogenase ; SDH, succinate dehydrogenase ; $\dot{V}O_2$ max, maximal oxygen uptake.

Introduction

Eleutherococcus senticosus MAXIM, Araliaceae (ES), is distributed widely around the middle reaches of the river Amor flowing through Russia and China, on the Korean peninsula and in east Hokkaido of Japan. Extract from ES has been used to improve physical health.[1] There are several papers[2-8] reporting pharmacological activities of ES extract. Nishibe *et al.*[3] reported that aqueous extract from the stem bark of ES showed an activity to reduce fatigue. Takeda[6] demonstrated that aqueous extract from the stem bark of ES reduced the consumption of hepatic glycogen in rats subjected to exhaustive swimming exercise and increased the duration time of the exer-

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cise. Asano *et al.*[7] and others[8] reported that ethanol extract from the root of ES increased the maximal oxygen uptake (VO_2 max) and oxygen puls max in human, suggesting that the extract enhanced the cardiac function and the oxygen metabolism in tissues and organs. Thus, it is very likely that extract from ES has the activity to improve aerobic metabolism, although the precise mechanism is unknown.

VO_2 max is generally thought to be a good indicator for the aerobic capacity of humans. It is known that the value of VO_2 max is affected by age and daily physical exercise.[9-11] It is known that the capacity for physical exercise correlated well with the oxidative enzyme activity involved in TCA cycle of skeletal muscle.[12-14] The enhancement of the metabolic capacity for producing energy in skeletal muscle probably results in the increase of VO_2 max.

In the present paper, we examined the effect of aqueous extract from the root of ES on activity of TCA cycle-related oxidative enzymes including succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) in skeletal muscle of mice, in relation to its VO_2 max increasing activity and anti-fatigue activity.

Materials and Methods

Animals : Male ICR mice (3 weeks of age, 10 to 12 g of body weight) and ddY mice (12 weeks of age, 35 to 36 g of body weight) were obtained from Japan SLC. Inc. (Hamamatsu, Japan). They were maintained with free access to solid rodent chow (CE-2 : Nihon Clea, Japan) and given water in a room under a 12 h light-dark cycle, at a temperature of 22±1°C and a relative humidity of 60±5%. ICR mice and ddY mice were acclimated to the environment for two weeks and one week before the experiment, respectively.

Drug : The root of *Eleutherococcus senticosus* MAXIM, Araliaceae (producted in the northeast region of China) was obtained from Matsuura Yakugyo Co. Ltd. (Nagoya, Japan). The sliced root (1 kg) was extracted with 2 ℓ of boiling water for 10 hours. This extraction procedure was repeated 5 times. The resulting extracts were pooled and evaporated in vacuo. The aqueous extract of *Eleutherococcus senticosus* (AERES) was suspended in distilled water and given to mice *p.o.* in a volume of 0.1 ml/10 g body weight in an appropriate concentration for the defined dose.

Experimental procedures :

Long term administration of AERES : ICR mice were given AERES *p.o.* at 170 mg/kg per day (6 days/week) for 9 weeks starting at 5 weeks of age. Control mice were given water (0.1 ml/10 g body weight) *p.o.*. The mice were sacrificed by bleeding under deep anesthesia with ether at 8, 10, 12, 14 and 19 weeks of age (5 weeks after the final administration) for measuring oxidative enzyme activities in gastrocnemius muscle.

Short term administration of AERES : ddY mice (13 weeks of age) were given AERES *p.o.* at 170 mg/kg per day for 10 consecutive days. The control mice were given water (0.1 ml/10 g of body weight) *p.o.*. The mice were sacrificed by bleeding under deep anesthesia with ether on the day after the final treatment with AERES for measuring the enzyme activities in gastrocnemius and tibialis anterior muscles.

Measurement of oxidative enzyme activity in skeletal muscle : Gastrocnemius and/or tibialis anterior muscles were taken from the mice immediately after the killing and the muscles were frozen using acetone-dry ice. To measure the enzyme activities, the muscles were thawed and homogenized in 0.03 M phosphate buffer (pH 7.4) for 30 sec with Digital homogenizer (Iuchi Co., Ltd., Osaka, Japan). The homogenates were centrifuged at 2,500 rpm for 10 min. The supernatants were used for measuring the SDH activity and/or MDH activity. The SDH activity and the MDH activity were determined spectrophotometrically according to the methods of Cooperstein *et al.*[15] and Hehler *et al.*,[16] respectively. The protein contents of the supernatants were measured according to the method of Lowry *et al.*.[17]

Statistics : Results were statistically evaluated using the Student's *t*-test. Significance levels (p) of 0.05 and 0.01 were employed. Data were expressed as the mean±S.E..

Results

Effect of AERES long term administration on the body weight, and SDH and MDH activities in gastroc-

nemius muscles of mice

The body weights of the control mice at 5, 14 and 19 weeks of age were 18.0±0.6 g (n=25), 42.9±0.9 g (n=10) and 43.6±1.2 g (n=5), respectively. The AERES-long term administration scarcely affected the body weight gain (data not shown).

Fig. 1 shows the effect of the AERES-long term administration on SDH and MDH activities in gastrocnemius muscle of the mice. The administration increased significantly the SDH activity in the muscle of mice at 8, 12 and 14 weeks of age in comparison with those of the control mice. Even at 19 weeks of age, 5 weeks after the final administration of the extract, the SDH activity was higher than that of control mice by 43 %, although the difference did not reach statistical significance. The MDH activities in the muscle were 10-20 times higher than the SDH activity as a whole. The administration of AERES did not affect the MDH activity except for an increase at 12 weeks of age.

Effect of AERES-short term administration on SDH and MDH activities in gastrocnemius and tibialis anterior muscles of mice

Fig. 2 shows the effect of the AERES-short term administration on the SDH and MDH activities in gastrocnemius and tibialis anterior muscles of mice. The MDH activity was clearly higher than the SDH activity not only in the gastrocnemius muscle but also in tibialis anterior muscle. The administration of AERES increased the SDH activities in both muscles

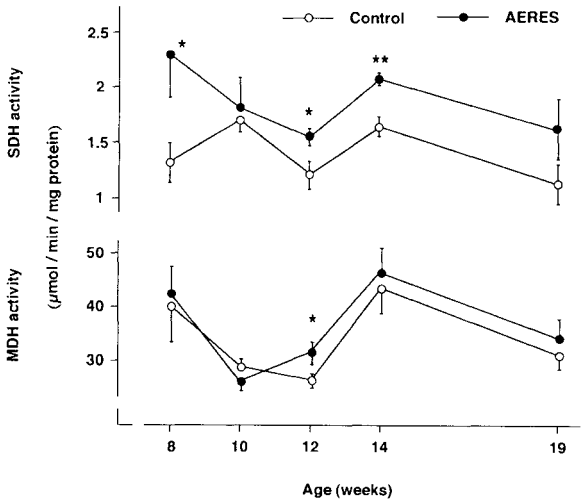

Fig. 1 Effect of long term administration of AERES on succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities in gastrocnemius muscle of mice. The extract was given *p.o.* at a dose of 170 mg/kg for 9 weeks starting at 5 weeks of age. Each point indicates the mean±S.E. of 5 mice. Statistically significant difference from the control at $*p<0.05$ and $**p<0.01$, respectively. AERES : aqueous extract of *Eleutherococcus senticosus*.

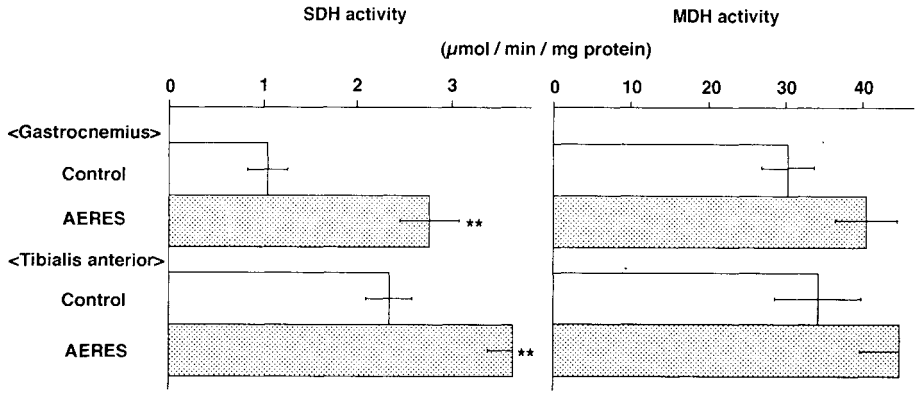

Fig. 2 Effect of short term administration of AERES on succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities in gastrocnemius and tibialis anterior muscles of mice. The extract was given *p.o.* at a dose of 170 mg/kg per day for 10 consecutive days starting at 13 weeks of age. Each column and horizontal bar represents the mean±S.E. of 8 mice. Statistically significant difference from the control at $**p<0.01$. AERES : aqueous extract of *Eleutherococcus senticosus*, Gastrocnemius : Muscle gastrocnemius, Tibialis anterior : Muscle tibialis anterior.

of gastrocnemius and tibialis anterior significantly. On the other hand, the administration showed only a tendency to increase the MDH activities in both muscles of gastrocnemius and tibialis anterior.

Discussion

Asano *et al.*[7] administered ethanol extract from the root of ES at a dose of 1 g per person to investigate the effect of the extract on physical working capacity and reported that the extract enhanced VO_2 max in humans. They speculated an increase of number of mitochondria as the action mechanism of the extract to increase energy metabolism of the muscles. In the present study, we used aqueous extract of ES, AERES. Mice were given *p.o.* AERES at a dose of 170 mg/kg which corresponded to the human dose (1 g/60 kg) used in the study of Asano *et al.*,[7] although there was a difference of aqueous extract and ethanol extract. AERES did not affect the body weight gain of the mice not only by the short term administration (data not shown) but also by the long term administration. The long term administration of AERES to mice starting at the infant stage (5 weeks of age) increased the SDH activity in the gastrocnemius muscle, although the treatment scarcely affected the MDH activity except the significant increase at 12 weeks of age.

It is reported that protein weight-relative activity of oxidative enzymes in skeletal muscles decreases during the growth period of animals, owing to rapid increase of the muscle protein.[18, 19] The decrease of the MDH activity observed at 10 and 12 weeks of age was probably dependent on the growth of mice. Therefore, in the next experiment to examine the effect of short term administration of AERES on the oxidative enzyme activities, we used 13 week old mice to avoid the effect of their growth on the activities. Again, the short term administration increased significantly the SDH activity in both muscles of gastrocnemius and tibialis anterior, but did not much increase the MDH activity.

We have not known the reason why AERES enhanced the SDH activity but not the MDH activity. The MDH activity was higher than the SDH activity in both muscles of gastrocnemius and tibialis anterior in agreement with the results reported by Suominen and Heikkinen for humans[20] and Takekura *et al.* for rats.[21] The higher background level of MDH might be the reason why AERES did not much affect the MDH activity. It is also known that there are three kinds of muscle fibers including slow-twitch oxidative (SO), fast-twitch-oxidative-glycolytic (FOG) and fast-twitch glycolytic (FG) fibers. Takekura *et al.*[21] reported that exercise of rat increased SDH activity but not MDH activity in FOG fiber-rich muscles, in contrast that the exercise increased both SDH and MDH activites in SO fiber-rich muscles. We examined here FOG fiber-rich muscles of gastrocnemius and tibialis anterior. This might be another reason why AERES did not increase the MDH activity while the extract increased SDH activity. These points remain to be studied in future, although the results obtained here at least for the SDH activity suggest that AERES enhances aerobic metabolism of skeletal muscles. This might be a mechanism of ES extract for the increasing activity of VO_2 max in humans which have been reported by Asano *et al.*,[7] although they used ethanol extract and we used aqueous extract.

The long term administration of AERES showed a tendency to increase SDH activity even at 5 weeks after the final administration. It is, therefore, likely that AERES increases the number of mitochondria rather than enhances the enzyme activity itself. We have not yet examined whether the AERES increases the number of mitochondria in skeletal muscles. This point also remains to be studied in future as a mechanism of AERES action.

In conclusion, it is suggested that AERES enhances the rate of aerobic metabolism through increasing the SDH activity of skeletal muscles.

和文抄録

エゾウコギの熱水抽出エキス（AERES）の長期間投与および短期間投与がマウスの骨格筋酸化系酵素のコハク酸脱水素酵素（SDH）活性およびリンゴ酸脱水素酵素（MDH）活性に及ぼす影響について検討した。長期間投与実験は，5週齢のICR系マウスを用いて1日170 mg/kgのAERESを週6日間，9週間にわたって経口投与し，8，10，12，14および19週齢時にそれぞれ剖検した。AERESを投与したマウス（AERES投与群）の腓腹筋の

SDH 活性は，対照群と比較して 8，12 および 14 週齢時で有意に増加した。AERES の最終投与から 5 週間後において，AERES 投与群の SDH 活性は対照群と比較して，高値傾向にあった。MDH 活性は 12 週齢時において有意に高値を示した。短期間投与実験は，13 週齢の ddY 系マウスを用いて 1 日 170 mg/kg の AERES を 10 日間連日経口投与した。AERES 投与群の腓腹筋および前脛骨筋の SDH 活性は，対照群と比較して有意に増加した。以上の結果から，AERES はマウスの骨格筋酸化系酵素活性を高め，生体内の有気的代謝機能を向上させることが示唆された。

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