Direct Effects of High Concentrations of Dimethylsulfoniopropionate, Vitamin E and Ferulic Acid on the Senility of Aged Scenescence-Accelerated Mouse (SAMP8)

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Summary The effects of high concentrations of dimethylsulfoniopropionate (DMSP), vitamin E and ferulic acid solutions on the aging of male and female scenescence-accelerated mouse (SAMP8) at the age of 50 wk were examined by direct supplementation to their stomachs twice a week for 28–30 d. The addition of 1 mL of DMSP, vitamin E and ferulic acid solutions (2% each) to the male and female mice in each group in this order rather elevated their growth and significantly suppressed their total grading score and loss of learning and memory with increasing rearing times for the short experimental period. However, there were no significant differences found between the male and female mice during the experimental period. The antioxidant and hormonal actions for the effects of the test compounds on the aged SAMP8 were possibly considered.

Key Words scenescence-accelerated mice, dimethylsulfoniopropionate, vitamin E, ferulic acid, aging, loss of learning and memory

Recently, various effective medicines for a number of diseases have been investigated and developed, which have allowed people to live longer and thus increased the number of senior citizens in Japan (1). There then occurs a social problem in that we have to ensure the health and comfortable life of our senior communities. I then aimed to obtain clues to delay, prevent and/or ameliorate various aging phenomena of humans in the present experiments.

All the scenescence-accelerated mice (SAM) were been derived from the parent strain, AKR/J (2). Among them, SAMP8 is known to be especially characteristic for the loss of learning and memory, immune dysfunction, and earlier aging and mortality (2). As the aging phenomena of SAMP8 are very similar to those in humans (2, 3), the SAMP8 has been one of the model animals for aged people and a number of investigations on senility with the SAM (4–10) has thus been performed.

The effects of dimethylsulfoniopropionate (DMSP), which is proven to widely occur in green sea algae, on various aquatic and terrestrial animals have been studied (11). The results indicated that DMSP stimulates the feeding, growth, moving ability, molting or metamorphosis of the animals (12). Especially, this compound proved to promote the moving ability of rats (13), and to prevent some diseases in rats (14, 15). Moreover, noticeable results have shown that a natural food stuff (green sea algae) (16) and its component (DMSP) (17, 18) delays and/or improves various senile phenomena in SAMP8.

There is a strong possibility that oxidative stress accelerates the aging of people (19–21). The effects of vitamin E and ferulic acid, being natural antioxidants, and DMSP on the aging of old male and female SAMP8 at the age of 50 wk were then compared for a short period.

MATERIALS AND METHODS

The chemicals, vitamin E (α-tocopherol) and ferulic acid, were purchased from Wako Pure Chemicals Co., Ltd., Japan. DMSP was synthesized by refluxing equimolar amounts of 3-bromopropionate and dimethylsulfoxide at 20°C for 22 h. The mixture was washed with ethylether under ice-cold conditions and crystallized from methanol.

The strains of the male and female scenescence-accelerated mouse, SAMP8/Ta (SAMP8), were kindly provided by Takeda Pharmaceutical Co., Ltd., Japan, and proliferated at a temperature of 23°C and relative humidity of 60% at intervals of 12 h-light and 12 h-dark in a pathogen-free rearing room. The male and female SAMP8 mice were fed distilled water and solid natural diets ("M," Oriental Yeast Co., Ltd., Japan) during the experimental period. The test mice were divided into four groups of five mice each at the age of 50 wk. One milliliter of the saline solution (control), 2% DMSP, vitamin E or ferulic acid solution dissolved or miscible in saline solution was employed as the test solution. The solutions were heated to body temperature and directly added to the stomachs of the test mice twice a week using a sterilized zonde (Φ 0.5 mm × 8 cm) and a syringe (1-mL volume), except that 0.02 g of oily authentic vitamin E solution and then 0.98 mL of saline solution were injected for the addition of the vitamin E solution. The body weights were individually measured at specified times. Each grading score of the aging mice was
Fig. 1. Effects of DMSP, vitamin E and ferulic acid on the growth of aged male SAMP8. One milliliter each of 2% DMSP, vitamin E and ferulic acid solutions was directly supplemented twice a week into the stomachs of male SAMP8 at the age of 50 wk and reared for 31 d. The values are shown as means±SD (n=5). The body weights of the male mice in the control, DMSP, vitamin E and ferulic acid groups at the start of the experiments were 23.7±0.74, 25.2±0.70, 25.1±0.80, and 25.9±1.02 g (means±SD, n=5), respectively. The marks, □, △, ○, and ◦, show the supplementation of the control, ferulic acid, vitamin E and DMSP solution, respectively. The symbols * and † exhibit significant differences (p<0.05) from the control and the vitamin E values at the same rearing time, respectively. For experimental conditions, see the Materials and Methods section for details.

Fig. 2. Effects of DMSP, vitamin E and ferulic acid on the growth of aged female SAMP8. The experimental conditions were the same as those in Fig. 1. The body weights of the female mice in the control, DMSP, vitamin E and ferulic acid groups at the start of the experiments were 24.5±1.46, 25.2±0.67, 24.0±1.25 and 25.2±0.38 g (means±SD, n=5), respectively. For experimental conditions, see the Materials and Methods section for details.

The statistical analyses were performed using ANOVA and Fisher’s PLSD tests.

RESULTS

Effects of direct administration of high concentrations of DMSP, vitamin E, and ferulate solutions on the growth of aged male and female SAMP8

The effects of the direct supplementation of 2% DMSP, vitamin E and ferulate solutions on the growth of male SAMP8 at the age of 50 wk were examined for 31 d. These results are shown in Fig. 1. The growth in the aged mice of the control rapidly decreased with the rearing times. In contrast, the growth in the mice of the DMSP and vitamin E group increased fairly well, although the former compound showed slightly higher stimulation compared to the latter during the experimental period. The growth in the group mice administered ferulate was lower than that of the DMSP and vitamin E groups, but much higher than that of the control group.

The same experiments as above were done with the aged SAMP8 females. The results are shown in Fig. 2, which showed the very same behaviors as those in Fig. 1, but the growth in the DMSP group mice was slightly but continuously higher than that in the vitamin E group mice during the middle and later stages of

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counted based on the individual senescence scores (numbering each item of reactivity, passivity, glossiness, coarseness, loss of hair, ulcers of skin, periophthalmic lesion, corneal opacity, corneal ulcers, cataracts and lordokyphosis from 0 to 4 or 5 (22)) and totaled, then expressed in terms of the total grading score.

The loss of learning and memory with aging was estimated by step-through experiments with equipment (DC-6 V 300 mA, Sony AC Adapter AC-9) previously reported (23), in which the test mouse is electrically stimulated just when the mouse moves from the copper mesh plate in the remaining light compartment into the same plate in the neighboring dark compartment. In the experiments, the five mice were simultaneously measured. The time (s) of the test mouse remaining in the light compartment after removing the wall between the light and the dark compartments is expressed as the criterion for assessing the learning and memory. Care and treatment of the experimental animals were in accordance with the guidelines of the Animal Center of
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Fig. 3. Effects of DMSP, vitamin E and ferulic acid on the total grading score of aged male SAMP8. The experimental conditions were the same as in Fig. 1. The total grading score is expressed in terms of the total number assessed from the 11 aging items. The total grading score of the male mice in the control, DMSP, vitamin E and ferulic acid groups at the start of the experiments was 7.3, 7.7, 7.5 and 7.7, respectively. For experimental conditions, see the Materials and Methods section for details.

Fig. 4. Effects of DMSP, vitamin E and ferulic acid on the total grading score of aged female SAMP8. The experimental conditions were the same as in Figs. 1 and 3. The total grading score of the female mice in the control, DMSP, vitamin E and ferulic acid groups at the start of the experiments 3.0, 7.0, 7.3, 7.0 and 7.3, respectively. For experimental conditions, see the Materials and Methods section for details.

Fig. 5. Effects of DMSP, vitamin E and ferulic acid on the loss of learning and memory of aged male SAMP8. The loss of learning and memory was expressed in terms of the remaining time (s) a test mouse stays in the lighted compartment of just after pulling up the wall between the lighted and the dark compartments. The experimental conditions were the same as in Fig. 1, except for the examination period of 28 d. For experimental conditions see the Materials and Methods section for details.

Effects of direct administration of high concentrations of DMSP, vitamin E, and ferulate solutions on the total grading score of aged male and female SAMP8

The effects of the direct supplementation of 2% DMSP, vitamin E and ferulate solutions on the total grading score of aged male SAMP8 at the age of 50 wk were examined for 31 d. These results are shown in Fig. 3. The total grading score of the control group mice significantly increased during the short rearing period. In contrast, the supplementations of DMSP and vitamin E much more effectively suppressed the total grading score of the mice than did that of the control solution, although the total grading score in the group of mice given DMSP was slightly but continuously lower than that in the vitamin E group mice during the short experimental period. The total grading score in the group of mice given ferulate was much more effective than that in the control group mice, but less effective than those in the DMSP and vitamin E group mice during the experimental period.

The same experiments as described above were performed with the aged female mice. These results are shown in Fig. 4. Also, in the case of female mice, the DMSP and vitamin E group mice and, to a lesser extent, the ferulate group showed much stronger suppressive effects on the total grading score for their short life spans.
Effects of direct administration of high concentrations of DMSP, vitamin E, and ferulate solutions on the loss of learning and memory of aged male and female SAMP8

The effects of the direct supplementation of 2% DMSP, vitamin E and ferulate solutions on the loss of learning and memory of male SAMP8 at the age of 50 wk were examined for 28 d. These results are shown in Fig. 5. The supplementation of DMSP and vitamin E exhibited significant suppressive effects on the loss of learning and memory, showing the same behaviors during the short rearing times. The administration of ferulate had a better effect than did that of the control solution during the experimental period.

The same experiments as above were made with aged female SAMP8. These results are shown in Fig. 6. The effects of the test compounds were the same as in Fig. 5 (in the male mice), in which DMSP and vitamin E, and to a lesser extent, the ferulate exerted remarkable suppressive effects on the loss of learning and memory of the aged mice during the experimental period.

**DISCUSSION**

A tertiary sulfonium compound, DMSP, has been proven to be largely contained in green sea algae (11), and to be easily degraded to produce the sea smell on the sea shore, dimethylsulide (24, 25). DMSP had previously been proven only to play roles as an osmoregulant (compatible solutes) (26–28) and a cryoprotectant (29, 30). However, the results of previous experiments have shown that DMSP stimulates the feeding, growth, body movement, the molt and/or metamorphosis of aquatic animals, fresh and marine fish, crustaceans, shellfish and amphibians (12). The compound was further determined to activate the moving ability of rats (13) and chickens (31), to relieve some environmental stresses from fish (32) and to suppress stress-induced gastric ulcers in rats (14). In addition, the orally administered experiments of DMSP to SAMR1 (the control mice against SAMP8) and SAMP8 over their life spans revealed that DMSP very effectively prevents and/or ameliorates senility along with extending the lives of both the male and female mice in both strains, especially SAMP8 (18). Thus, a number of favorable effects of DMSP on aquatic and terrestrial animals have been found.

Vitamin E (α-tocopherol) is well known to be a natural antioxidant (33–35), and has been reported to exert ameliorating effects on various diseases, ovarian cancer (36), cardiovascular disease (37), diabetes mellitus (38), atherosclerosis (39), etc. In contrast, a natural antioxidant, ferulic acid (40, 41), is proven to be contained in grains (42) and exhibit inhibitory effects on the oxidation of neuronal cells (43) and LDL (44), carcinogenesis (45), etc.

The senile phenomenon of SAMP8 is very similar to the senile dementia and Alzheimer disease of humans, especially with regard to the characteristics of immune dysfunction, early aging and death along with the loss of learning and memory (2, 3). The various senilis in a group of mice begin to occur at about the age of 16 wk (22). Accordingly, the senility of the mice aged 50 wk highly proceeded as seen in the results of the total grading score of the control group mice in the present experiments (Figs. 3 and 4) and in previous reports (2, 46). To clearly assess the effects of test compounds on the aging of the male and female SAMP8, the effects of DMSP, vitamin E (α-tocopherol) and ferulic acid on the senile phenomenon of aged male and female SAMP8 at the age of 50 wk by directly adding high concentrations of the compounds to their stomachs were evaluated. In the preliminary experiments, the direct addition (1 mL) of more than 2% of the vitamin E solution proved to decrease the moving ability of the test mice and to sometimes cause diarrhea, while the direct administration (1 mL) of more than 2% of the DMSP and ferulic acid solutions caused no abnormal behaviors in the test mice. On the contrary, the supplementation of more than 1 mL (1 mouse) of the test solutions was frequently disgorged by the test mice. Thus, the test compounds used in the trial of the direct supplementation were done at the volume of 1 mL and the concentration of 2%.

The supplementation of DMSP clearly exhibited noticeable increasing effects on body weight and afforded significant suppressive effects on the total grading score and the loss of learning and memory of the aged male and female SAMP8 against that of the control solution during the short experimental period. Therefore, the results in the present experiments demonstrate that DMSP exerts not only significant suppressive effects on the senility of the adult (35 wk-old) (17).
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and aged (50 wk-old) male and female SAMP8, but also on these mice regarding their life spans (more than 80 wk-old) (18).

There are reports that vitamin E does not exert a favorable effect on the aging of animals (47–50), whereas the dietary and oral supplementation of vitamin E to rats and human is reported to exhibit suppressive effects on lipid oxidation (51, 52). In the present experiments, the administration of vitamin E proved to exert a clear-cut improvement effect on the senility of aged male and female SAMP8 by the direct supplementation method, showing slightly lower effects on the body weight of male and female SAMP8 and the total grading score of male SAMP8 compared to those undergoing direct supplementation of DMSP. The effects of vitamin E were also evidently confirmed in the male and female SAMP8 at the age of 30 wk by the same direct supplementation methods, although the direct addition of DMSP was much more effective in the total grading score as compared to vitamin E (17).

The supplementation of ferulic acid induced effects that were not as favorable as those of DMSP and vitamin E on the senile phenomenon of the aged male and female SAMP8 in the high and direct doses, although the administration of ferulic acid showed much more favorable effects on the senility of both mice and rats that of the control solution. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) also bears a phenolic hydroxyl group on the molecule-like vitamin E (53), which may suggest that the effects of the compound on the aged male and female SAMP8 are the same as those of vitamin E. However, the effects were weaker than those of vitamin E in the present and previous experiments (17). This may be attributable to the formation of a linear dimer and an oligomer of ferulic acid by hydrogen bond-ding (masking phenolic hydroxyl group) (54), which makes the antioxidant action weak and/or negligible. The formation of these products is well known in the relations of 3-hydroxybenzoic acid (non-active) to 3-hydroxybenzoic acid (salicylic acid, active) and of 1-phenyl-3-methyl-5-pyrazolone (non-active) to 1-phenyl-2,3-dimethyl-5-pyrazolone (antipyrine, active) (54).

From the results obtained and previous reports, DMSP possibly functions as an antioxidant (melanins and oxidative products) (55, 56), the methylated bioactive compounds by DMSP (57), an activator of the immune power (58) and/or as a stimulant of biosynthesis and excretion of hormones, catecholamines in rats and mice (Nakajima K, unpublished data). Accordingly, the effects of DMSP on the aging of male and female mice, large and small, are considered to be the result of the participation of all the effects of DMSP. However, among these effects, the recovering effects from the loss of learning and memory by DMSP appear to be very significant because the loss of learning and memory is believed to be a critical problem for the not only animals, but humans as well. Our preliminary experiments indicated that the administration of DMSP accumulates catecholamines (epinephrine, norepinephrine, dopamine) earlier in the brain than in the liver of test rats (Nakajima K, unpublished data). This finding may thus indicate that the recovering effects of DMSP from the loss of learning and memory with aging are attributable to the activation of the adrenergic nervous system (59–61), probably accompanied by the augmentation of the cholinergic and glutamatergic nervous system in the central nervous system (59, 62–64).

The action mechanisms of DMSP in the aging of animals still remain to be determined.

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