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Amelioratory Effect of Dietary Ingestion of Lycopene and Tomato Rich in Lycopene on Learning Impairment in Senescence-Accelerated Mice (SAMP8)

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We previously reported that the dietary ingestion of red bell pepper (*Capsicum annuum* L.) which contained a large amount of antioxidative carotenoids ameliorated the age-related phenomena of learning disorder and degradation of hair glossiness in the senescence-accelerated mouse (SAM). As lycopene is one of the most popular carotenoids in human serum and exists in abundance in tomato, we examined the effects of dietary lycopene and tomato rich in lycopene on the age-related disorders in SAMP8//Kgm, a mouse model of accelerated decline in learning and memory, and control SAMR1//Kgm mice. SAMP8 mice that received a diet containing 0.02% (w/w) lycopene or 20% (w/w) tomato powder showed much better acquisition in passive avoidance tasks than those given the control diet. Dietary lycopene and tomato had no effect on the ability of learning and memory in SAMR1 mice. Those observations indicated that the dietary ingestion of lycopene in tomato ameliorated the learning impairment in SAMP8.

Keywords: senescence-accelerated mouse (SAM), lycopene, learning, memory, passive avoidance, tomato

The senescence-accelerated mouse (SAM) was established in Kyoto University as a murine model of accelerated senescence (Takeda *et al*., 1981). SAM strains consist of the senile-prone strain (SAMP) and its resistant strain (SAMR). SAMP strains are known to exhibit phenomena of human aging such as amyloidosis (Matsumura *et al*., 1982; Higuchi *et al*., 1986), osteoporosis (Matsushita *et al*., 1986), and cataracts (Hosokawa *et al*., 1984). SAMP8 separated from the SAMP strain shows significant impairments in various learning and memory tasks as compared with SAMR1 (Miyamoto *et al*., 1986; Yagi *et al*., 1988), especially in passive avoidance tasks (Miyamoto, 1997) and in spatial tasks escaping from an adverse situation (Nishiyama *et al*., 1997).

Free radicals are the most important environmental factors in several neuronal degeneration processes as well as in age-related physiological decline (Harman, 1956, 1981). Many alterations similar to the pathology of aged humans, for example, spongy degeneration (Yagi *et al*., 1989), neuronal cell loss (Kawamata *et al*., 1990), and gliosis (Nomura & Okuma, 1999) have been reported in the SAMP8 brain, and oxidative stress has been shown to be involved in these alterations (Manganaro *et al*., 1995; Schulz *et al*., 1997; Voigtlander *et al*., 2001). Thus, supplementation with antioxidants is suggested to ameliorate age-related disorders in SAMP8, and it has been reported that administration of spin-trap *N*-tert-alpha-phenyl-butylnitrone (Edamatsu *et al*., 1995) or antioxidative aged garlic extract (Moriguchi *et al*., 1996) prolonged the life span and improved learning disorders in SAMP8.

We reported previously that dietary ingestion of red bell pepper (*Capsicum annuum* L.) ameliorated the age-related alterations of SAMP8//Kgm (Suganuma *et al*., 1999) and that the beneficial effect was due to capsanthin, an antioxidative carotenoid. Tomato also contains a large quantity of the antioxidative carotenoid, lycopene, which has been reported to prevent many kinds of degenerative diseases typified by cancer (Giovannucci, 1999). We evaluated the effect of dietary tomato and lycopene on age-related alterations in SAMP8 in this study.

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Materials and Methods

Animals and diets SAMP8 and SAMR1 mice were originally obtained from The Council for SAM Research, and bred under conventional conditions. The mice were housed in a temperature (23–25˚C)- and humidity (40–60%)-controlled room with a 12-h light and dark cycle (lights on from 06:00 to 18:00). They were given a commercially available diet (CE-2, Japan CLEA, Tokyo) until 6 weeks old. Thereafter, they received experimental diets in place of the common diet continuously for three months. Animals were given free access to water and the powdered diets throughout the experiments. The control diet consisted of the following (g/kg): casein, 200; β-cornstarch, 397.486; α-cornstarch, 132; sucrose, 100; soybean oil, 70; cellulose powder, 50; AIN-93G mineral mixture, 35; AIN-93 vitamin mixture, 10; L-cystine, 3; choline bitartrate, 2.5; *t*-butyl-hydroquinone, 0.014. The composition of the diets containing lycopene or lyophilized powder of tomato resembled the control diet except that 0.02% (w/w) lycopene or 20% (w/w) lyophilized powder of tomato replaced the same weight of β-cornstarch. All components of the diets except lycopene and the tomato powder were purchased from Oriental Yeast, Tokyo. Lycopene was extracted from tomato paste (TAT, Istanbul, Turkey) using hexane, acetonitrile, ethanol, and toluene (10:7:6:7, v/v/v/v) after saponification with KOH, concentrated under reduced pressure and purified by HPLC (Model 575, GL Science, Tokyo) with reverse phase column (ϕ100×500 mm, ODS-ST-C, Soken Chemical & Engineering, Tokyo). The tomato powder was obtained by lyophilizing the tomato paste. The experimental procedures used in

this study met the standards set forth in the Guidelines for the Care and Use of Laboratory Animals of the Experimental Animal Facility, the Japanese Society of Nutrition and Food Science.

Grouping in Experiment 1 Twelve male SAMP8//Kgm mice were assigned to 2 groups of 6 mice each and they received the control diet or the diet added with 20% (w/w) lyophilized powder of tomato. The tomato powder contained 0.15% lycopene.

Grouping in Experiment 2 We divided the male mice into 4 groups of 6 mice each and they received the control diet (P8-C, R1-C) and the diet containing 0.02% (w/w) lycopene (P8-L, R1-L). The lycopene level in the experimental diet was determined by a preliminary experiment to assure its accumulation in the liver was equal to that in experiment 1.

Grading score The degree of senescence was evaluated by a grading score system (Hosokawa, 1990) every month during the feeding experiment. The grading score was expressed as summation of the scores of 11 items: reactivity, passivity, hair glossiness, hair coarseness, loss of hair, skin ulcer, periophthalmic lesion, corneal opacity, corneal ulcer, cataract and lordkyphosis.

Passive avoidance The learning and memory performance of mice were assessed at the end of the feeding experiment by a two-compartment step-through passive avoidance apparatus (Ugo Basile, VA, Italy). An illuminated light chamber (110×210×212 mm) was connected to a dark chamber (222×210×212 mm) equipped with a grid floor, and the two chambers were separated by a door (70×70 mm). In acquisition trials, a mouse was placed in the light chamber and the time before it entered the dark chamber was recorded. As soon as the mouse entered the dark compartment, the door was closed and a 1.2 mA AC scrambled footshock was applied to the floor grid for 3 s. The first retention test was conducted 24 h after the initial acquisition trial, and two subsequent trials were performed at a 24 h interval. The mice entering the dark compartment were given footshocks repeatedly except on the last day.

Morris water maze test To evaluate the ability of spatial recognition of the mice in experiment 2, the Morris water maze test was performed following the method of Zhang *et al*. (1994). The elapsed time for a mouse to climb onto the platform (escape latency) was recorded by a color video tracking system (CompACT VAS, Muromachi Kikai, Tokyo) and evaluated by a block of three trials. Each block of trials was repeated for five consecutive days. The platform was placed at a fixed quadrant and the mice were put into the pool from the center of the circumference of each of the other three quadrants. On the day following the last block, the platform was removed and the mouse was made to search for it as a memory retention test for 60 s. The number of times the animal crossed the area where the platform had been was taken as an indicator of memory retention.

Liver and brain concentrations of lycopene The assays of lycopene in the liver and cerebral cortex were performed by HPLC as previously described (Oshima *et al*., 1997). Briefly, the tissues were homogenized and saponified by addition of 60% KOH and 3% butyrated hydroxytoluene in ethanol, followed by heating at 50°C for 30 min, then extracted twice with hexane and dichloromethane (4:1, v/v). The supernatant was dried and reconstituted in a solvent mixture of methanol, acetonitrile, dichloromethane, and water (7:7:2:0.16, v/v/v/v) as a mobile phase for HPLC. Analyses were performed using a Shimadzu SPD-10AV spectrophotometric detector (Shimadzu, Kyoto) and a Lichlospher RP18-5 column (E. Merk, Darmstadt, Germany) at a flow rate of 1.0 ml/min.

Statistical analyses All data were expressed as means and SE. The mean avoidance time of the passive avoidance test and the escape latency of the Morris water maze test were analyzed using the Mann-Whitney U-test (Mann & Whitney, 1947) for comparison between each of the groups. Student's *t*-test was used in experiment 1 and ANOVA followed by Tukey's test was employed in experiment 2 (Spjotvoll & Stoline, 1973). These analyses were performed using commercially available computer software (Visual Stat for Windows ver. 4.5J, Stat Soft, Tulsa, OK).

Results

Experiment 1 Table 1 shows the grading score, the body weight gain, the wet-weight of liver and the liver levels of lyco-

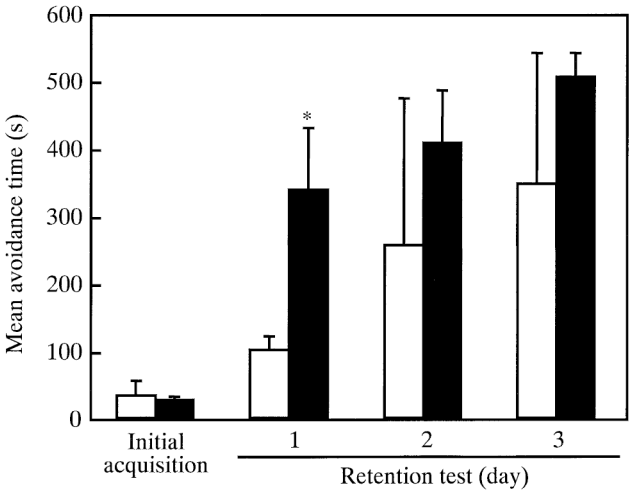

Fig. 1. Effect of dietary tomato on passive avoidance performance in SAMP8. The latency before footshock on passive avoidance response in SAMP8 fed the control diet (open columns) and the diet containing 20% tomato powder (closed columns) are shown. When the mouse remained in the light chamber over 9 min, the trial was ended. The values are expressed as means and SE (n=6). Significantly different from control; *p<0.05 (Student's *t*-test).

Table 1. Grading score, body weight gain, liver weight and lycopene levels in the liver in experiment 1.

Group	n	Mean Grading score at:				Body weight gain (g/3 months)	Liver weight (g)	Liver lycopene (μg/g wet tissue)
		Beginning	1 months	2 months	3 months			
Control	6	0.17±0.17	0.50±0.22	1.00±0.52	3.67±1.05	10.3±2.0	1.79±0.06	ND
Tomato	6	0.33±0.21	0.83±0.31	1.33±0.33	3.00±0.63	8.0±0.7	1.69±0.07	10.41±1.85

Values are shown as means±SE. ND: not detected.

Table 2. Grading score, body weight gain, liver weight and lycopene levels in the liver in experiment 2.

Group	n	Mean Grading score at:				Body weight gain (g/3 months)	Liver weight (g)	Liver lycopene (μg/g wet tissue)
		Beginning	1 months	2 months	3 months			
P8-C	6	1.13±0.23[a]	3.50±0.42[a]	4.88±0.52[a]	5.75±0.34[a]	7.6±0.9	1.75±0.19	ND
P8-L	6	1.13±0.23[a]	4.00±0.33[a]	5.75±0.37[a]	6.25±0.53[a]	8.1±0.6	1.82±0.04	14.89±2.52
R1-C	6	0.17±0.11[b]	0.67±0.14[b]	1.33±0.19[b]	2.50±0.16[b]	9.9±0.9	1.83±0.04	ND
R1-L	6	0.17±0.17[b]	0.50±0.22[b]	1.67±0.33[b]	2.67±0.24[b]	7.5±0.6	1.81±0.11	16.61±0.78

Values are shown as means±SE. Within a column, values with different superscripts are significantly different, $p<0.05$ (Tukey's test).

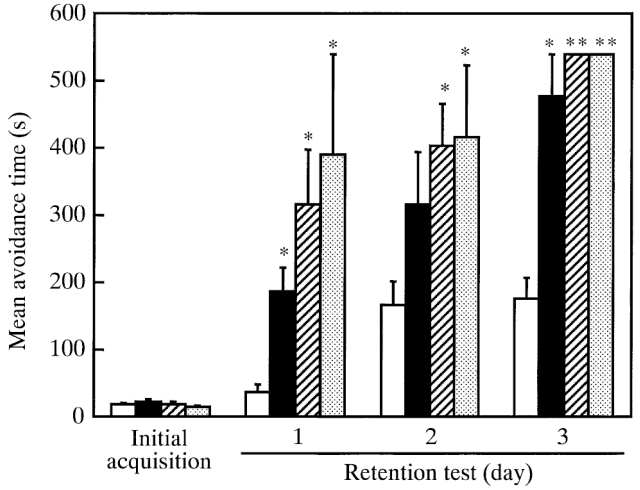

Fig. 2. Effect of lycopene on passive avoidance performance in SAM mice. The latency before footshock on passive avoidance response in each group is shown as mean and SE (n=6); open columns, P8-C; closed columns, P8-L; striped columns, R1-C; dotted columns, R1-L. When the mouse remained in the light chamber over 9 min, the trial was ended. Significantly different from control; *p<0.05, **p<0.01 (Mann-Whitney U-test, vs. P8-C).

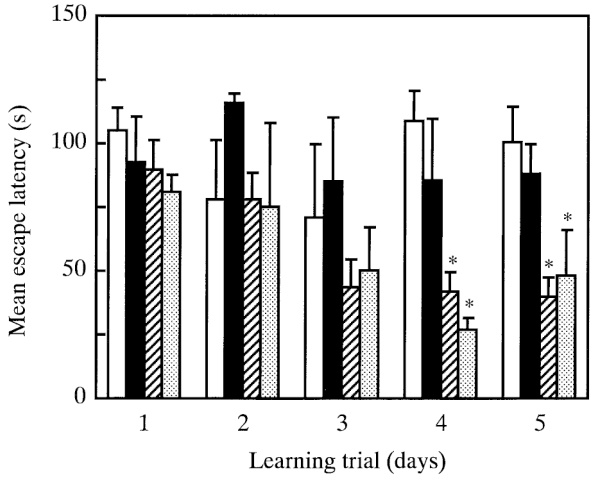

Fig. 3. Mean escape latency of SAM mice in Morris water maze test. The time for the mouse to climb onto the hidden platform is shown as the mean and SE (n=6); open columns, P8-C; closed columns, P8-L; striped columns, R1-C; dotted columns, R1-L. When the mouse could not get to the platform within 2 min, the trial was ended. Significantly different from control; *p<0.05, (Mann-Whitney U-test, vs. P8-C).

pene. Both groups showed an increase in the grading score during the feeding experiment but there was no statistical difference between them. Dietary ingestion with tomato did not affect the body weight gain or the wet-weight of liver. Lycopene was only detected in the liver of mice fed the tomato diet. The mean avoidance time in the step-through test is shown in Fig. 1. The escape latency of the mice given the tomato diet was longer than that of the control mice on day 2.

Experiment 2 Table 2 shows the grading score and body weight gain during the feeding experiment. The grading score of SAMP8//Kgm was significantly higher than that of SAMR1//Kgm throughout except in the beginning, but the feeding of lycopene had no effect on either grading score or body weight gain. There were no differences in wet-weight of liver among the 4 groups, and lycopene was not detected in the cerebral cortex (less than 50 ng/g wet tissue), although it was in the liver of mice fed the diet containing 0.02% (w/w) lycopene. The mean avoidance time in the step-through test is shown in Fig. 2. The mean latency of P8-C was significantly shorter than that of R1-C throughout the retention test (p<0.05, Mann-Whitney U-test). In the SAMP8 group, the diet containing 0.02% lycopene significantly prolonged the latency on days 1 and 3 (p<0.05) compared with the control diet, but no such effect was observed in the SAMR1 group. Figure 3 shows the escape latency in the Morris water maze test. In the SAMR1 group, the escape latency became shorter independent of the diet in an alternating succession of trials but no learning effect was observed in the SAMP8 group. The SAMR1 group was superior to the SAMP8 group in escape latency after day 3. The number of times crossing the goal area in free swimming on day 5 in each group was as follows: P8-C, 0.25±0.25; P8-L, 1.25±0.63; R1-C, 2.50±0.89; R1-L, 4.00±1.53. The number in the SAMR1 group was significantly larger than that in the SAMP8 group (p<0.05, Tukey's test), but supplementation with lycopene had no effect. No obvious effect of dietary lycopene was observed with regard to spatial learning performances.

Discussion

We found in this study that feeding a diet containing 0.02% lycopene or 20% lyophilized powder of tomato ameliorated the learning impairment in the passive avoidance task in SAMP8. This indicated that the beneficial effect of feeding tomato on learning ability in this group was mainly due to lycopene. After Harman (1956) put forward the theory that aging was due to free radicals, oxidative stress has been thought to be responsible for the majority of age-related alterations. Mitochondrial dysfunctions induced by an inefficient hyperactive state in the mitochondrial electron transport system with a concomitant increase in free electron defluxion was shown to be involved in the learning and memory impairments in SAMP8 (Nishikawa *et al*., 1998; Fujibayashi *et al*., 1998). Lycopene, a typical and the major carotenoid in tomato, was shown to be absorbed by feeding tomato

juice in humans (Sakamoto *et al*., 1994), and inhibited the oxidation of plasma low-density lipoprotein (Ojima *et al*., 1993). Narisawa *et al*. (1998) reported that *N*-methylnitrosourea-induced colon carcinogenesis in rats was prevented by dietary lycopene and tomato juice. We could not detect lycopene in cerebral cortex (less than 50 ng/g wet tissue) and there have been no previous reports showing its transport into the brain of rodents. But it was detected in the liver in this study and accumulations of β-carotene in the brain were reported in human (Mathews-Roth *et al*., 1976), bovine (Shi *et al*., 1991) and rat (Shapiro *et al*., 1984). These results led us to two hypotheses about the working point of lycopene. First, lycopene may be transported into the brain where it exerts beneficial effects on learning impairment in SAMP8. Second, lycopene cannot pass the blood brain barrier but may complement the endogenous antioxidative system and indirectly ameliorate the learning disorder in SAMP8.

Antioxidants also suppress age-related skin alterations. The importance of ascorbic acid and α-tocopherol was suggested by decreases of these antioxidants in skin levels with concomitant formation of lipid hydroperoxides during UV irradiation of murine skin, and the formation of squalene hydroperoxides in human skin upon UV exposure, respectively (Yamamoto, 2001). Carotenoids such as lycopene and β-carotene also attenuated the disorders elicited by UV irradiation (Ribaya-Mercado *et al*., 1995; Stahl *et al*., 2001). Although feeding red bell pepper attenuated the increase in the grading score in SAMP8 in our previous study, supplementation with lycopene or tomato could not do. As red bell pepper contains many antioxidants such as ascorbic acid and α-tocopherol (590, 17.6 μg/g, respectively) other than carotenoids (β-carotene, 13.2; capsanthin, 78.0 μg/g), its beneficial effect on the grading score might have been the synergy effect of these antioxidants.

In this study, SAMP8 was inferior to SAMR1 on the mean latency of the step-through type passive avoidance task and the escape latency of the Morris water maze task, and the ameliorative effect of feeding lycopene was shown in the former but not in the latter. This suggested that ingestion of lycopene slightly improved the learning ability in passive avoidance but had no effect on spatial perception. The mechanism by which lycopene elicits the beneficial effect in SAMP8 remains to be elucidated.

In conclusion, dietary ingestion of lycopene or tomato attenuated the age-related learning impairment in SAMP8. This beneficial effect may be the result of the antioxidative effect of lycopene. This is the first report to elucidate the beneficial effect of dietary ingestion of lycopene, one of the major carotenoids in human serum on learning disorders in SAM mice.

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