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Favorable Effects of Tea on Reducing the Cognitive Deficits and Brain Morphological Changes in Senescence-Accelerated Mice

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Summary The present study was carried out to explore the effects of oolong and green teas on improving the memory deficits and brain pathological changes in senescence accelerated-prone mice P8 (SAMP8). Six-month-old mice were supplied with oolong tea, green tea or water as the sole drinking fluid for 16 wk. The memory ability of mice was evaluated by passive and active avoidance tests, while the extent of the brain degeneration was measured by the spongiosis grades and the lipofuscin percentage in the hippocampus. The total grading score and serum biochemical levels were also measured. The results indicated that the mice supplemented with the oolong and green tea drinks reversed the cognitive impairment, lessened the spongy degeneration and lipofuscin, and increased the serum Trolox equivalent antioxidant capacity more than the control group. The total grading score of the oolong tea group was lower than that of the control group in male mice, whereas it did not differ among female groups. No differentiations in the concentrations of total cholesterol, triglyceride, glucose, iron or hemoglobin were observed among three drink groups. In conclusion, oolong and green teas could reduce the deteriorations of cognitive ability, brain degenerative changes and aging process in SAMP8, probably through the potent antioxidative activity of the tea.

Key Words oolong tea, green tea, learning and memory, brain degeneration

The impairment of the cognitive functions, such as learning and memory ability is considered to be one of the most striking impairments of the aging process. The free radical theory of aging shows promise of application, and oxidative damage has been found in all classes of organic molecules that are critical for maintaining neuronal structural and functional integrity. Dietary antioxidant components have received particular attention because their function in modulating oxidative stress associated with the aging process and many age-associated diseases (*1*). Several studies have revealed that age-dependent oxidative processes can be moderated by the application of various antioxidants, to improve the cerebellar physiology and the motor learning in aged rats (*2–5*).

Tea is the mostly widely consumed beverage worldwide and has become an important agricultural product (*6*). Beneficial health effects of tea have been demonstrated in both animal and human experiments. Polyphenols in the tea are powerful antioxidants that may decrease the risk of heart disease and are associated with the lower risk of specific types of cancer by diminishing the oxidation of LDL-cholesterol and the formation of oxidized metabolites of DNA (*7*). Yang and Landau (*6*) found that green tea, black tea and isolated tea polyphenols could scavenge the reactive oxygen and nitrogen species, and were able to lower the damage of cell membranes, protein and nucleic acids. Oolong tea was demonstrated to lower the atherogenic index and to increase the HDL-total cholesterol ratio, and epicatechin gallate and epigallocatechin gallate in the tea extracts may account for the hypocholesterolemic effect (*8*). Caffeine, another major component of tea, was demonstrated to inhibit the oxidative damage induced by the reactive species in the membrane, and was suggested to be a potent antioxidant for preventing lipid peroxidation (*9*). Caffeine has also been shown to exert protective effect against toxic components of radiation damage in cell culture systems (*10*).

Although the benefits contributed by antioxidants of tea have been demonstrated, the effect of tea on memory deficit and brain morphology is still unknown. The senescence accelerated-prone mice P8 (SAMP8) exhibited a remarkable age-related deterioration in learning and memory ability and showed definite responses when challenged with various learning and memory tasks such as passive avoidance, two-way active avoidance and water maze tests (*11, 12*). Thus, it is an excellent murine model for studying the deficits in learning

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and memory ability. The main purpose of this study was to examine the effects of oolong tea and green tea on the learning and memory ability and brain morphological changes in memory-deficient SAMP8.

MATERIALS AND METHODS

Animals, diets and fluid intakes. Six-month-old SAMP8 were used in this experiment. The mice were housed about 5 per cage under controlled environmental conditions (25±2°C, 65±5% relative humidity, 0700–1900 h lighting period). The experimental animals were divided into control (male, $n=30$; female, $n=30$), 1% oolong tea (male, $n=30$; female, $n=30$) and 1% green tea (male, $n=30$; female, $n=30$) groups, and allowed free access to chow diets. Oolong tea and green tea were supplemented to animals as the sole drinking fluid in the two experimental groups for 16 wk, while the control group was maintained on the same distilled deionized water that was used to brew the tea. Oolong tea was prepared by adding 100°C distilled deionized water to a glass container holding the oolong tea bags and prepared each morning, while the green tea was prepared by adding 70°C distilled deionized water. The tea bag was steeped for 20 min, and then removed. The tea solution was cooled to room temperature. Each serving of tea was prepared from 100 mL of water with 1 g oolong tea or green tea. The fluid and food intake of the mice in each cage was recorded every day and the value was divided by the number of the mice in that cage to represent the mean fluid and food intake for the mice in that cage. The body weights of the mice were ascertained weekly. This study protocol was approved by the Animal Research Ethics Committee at Providence University, Taichung,Taiwan.

The concentrations of caffeine, gallic acid, flavanols and polyphenols in oolong tea and green tea were analyzed by means of HPLC with UV detection at 280 nm (*13*). Their concentrations are listed in Table 1. The concentrations of caffeine, epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) in the oolong tea (220.8 μg/mL, 229.8 μg/mL and 46.4 μg/mL, respectively) were approximately half of those in the green tea (460.6 μg/mL, 444.7 μg/mL and 73.2 μg/mL, respectively), while the gallic acid, epicatechin (EP) and polymeric polyphenols concentrations in the oolong tea (22.3 μg/mL, 62.7 μg/mL and 194.5 μg/mL, respectively) were much higher than those in the green tea (0.3 μg/mL, 28.3 μg/mL and 48.7 μg/mL respectively). The concentrations of other polyphenols did not differ between the oolong and green tea.

Table 1. Composition of oolong tea and green tea.[1]

	Contents (μg/mL)	
	Oolong tea[2]	Green tea[3]
Gallic acid	22.3	0.3
Epigallocatechin (EGC)	223.0	268.7
Catechin (C)	15.8	11.0
Epicatechin (EP)	62.7	28.3
Epigallocatechin gallate (EGCG)	229.8	444.7
Gallocatechin gallate (GCG)	8.3	6.4
Epicatechin gallate (ECG)	46.4	73.2
Catechin gallate (CG)	1.0	2.5
Polymerized phenols	194.5	48.7
Caffeine	220.8	460.6

[1] Analyzed by the HPLC equipped with a detector with UV detection at 280 nm (*13*).
[2] 1 g oolong tea was steeped in 100 mL of 100°C distilled deionized water for 20 min.
[3] 1 g green tea was steeped in 100 mL of 70°C distilled deionized water for 20 min.

Evaluation of grading score. A grading score system was designed to represent the changes in the behavior and appearance of mice (*14*). The eleven categories, which included reactivity, passivity, glossiness, coarseness, hair loss, ulcer, periophthalmic lesions, cataract, corneal ulcer, corneal opacity and lordokyphosis, were evaluated by the same observer. In general, each category has five grades to represent the intensity of the changes. For example, grade 0 represents no particular changes and grade 4 represents the most severe changes. The details for each grade in each category are given elsewhere (*14*).

Memory evaluation. One week before the learning and memory examination, the mice were individually placed in cubic boxes with a length of 25 cm, and then the ambulatory activity of all the mice was measured for 10 min using a video activity monitor (E61-21 Coulbourn Instruments, Philadelphia, PA). Mice that failed to pass this test were excluded from the subsequent tests. Single-trial passive (step-through) and active (shuttle) avoidance tests were performed to evaluate the learning and memory ability of tested mice after being supplemented with the experimental drinks for 16 wk. Nearly half of the mice were exposed to the passive test, while the others were exposed to the active test. No mice were exposed to both memory tests.

The single-trial passive avoidance test used a shuttle box (35×17×20 cm: width×length×height, Coulbourn Instruments Model E10-15) that consisted of two equal compartments connected by a small opening (7.5×6.5 cm, Guillotine door, Coulbourn Instruments Model E10-15GD). One of the compartments was lighted while the other compartment was covered with a black semi-transparent plastic on the top. The floor of the box was a platform of steel rods. Each mouse exposed to this test was placed in the lighted compartment, and after a brief orientation period (10 s), the partition of the opening was raised to allow the mouse to explore the apparatus freely. Once the mouse was entered the dark compartment, the partition was lowered and three 0.5 mA electric shocks to the floor grids for 1 s each with 5 s interval were used as a negative reinforcement. After the negative reinforcement, each mouse was placed in the lighted compartment for 1, 2, 3 and 7 d, and the latency in entering the dark compartment (the time in seconds for the mouse to avoid the punishment by staying in the light compartment)

was recorded. The active (shuttle) avoidance test examined the successful avoidance response which was conducted if the tested mouse moved itself from one compartment to the other compartment within the shuttle box after being giving a conditional stimulus (CS), 10 s of tone and red, yellow and green light. If the tested mouse did not perform a successful avoidance response, an unconditional stimulus (UCS), a 0.3 mA, 5 s scrambled foot shock, would be given during the CS presentation. Each mouse received four daily sessions of a combination of 5 CS/UCS trials, a total of 20 trials each day, for 4 d. Between the sessions tested mice were allowed to rest for 15–20 min. The avoidance responses of tested mice were recorded automatically.

Biochemical parameters. After the learning and memory examination, the mice were anesthetized and then sacrificed for the subsequent tests. A blood sample was withdrawn and centrifuged at 1,200×*g* for 10 min, and then part of the serum sample was used to determine the total cholesterol, triglyceride, glucose, iron, and hemoglobin levels by an enzymatical method using an automatic analyzer (Synchron CX-7 systems, Beckman, USA) at a local analytical service center (Lian-Ming Co., Taichung, Taiwan). The liver tissues were diluted by a phosphate-buffered saline (80 mmol/L, pH 7.4), homogenized and centrifuged at 3,000×*g* for 10 min in a refrigerated high-speed centrifuge, and then the supernatant was collected. The Trolox equivalent antioxidant capacities (TEAC) of serum and liver samples were determined using an automatic analyzer (550 express, CIBA-Corning, USA) according to the method described by Miller et al. (*15*).

Morphology analyses. For examining the hippocampus morphology changes, the whole brains of the mice were immediately dissected after sacrifice and individually fixed in a 10% buffered neutral formalin solution for 1 wk. Brain regions at A, B, C and D-section were interval dissected using a mouse brain matrix (coronal slices model, Sunpoint 1 mm, Kent Scientific Corporation, CT) (*16*). Brain tissues were processed by histopathological techniques, sectioned at 5 μm thickness, and observed under a light microscope (Opticphot-2, Nikon, Tokyo, Japan). According to the description of Popesko et al. (*16*), the B section of the brain has the best performance for hippocampus. Therefore, part of the B-section of the brain hemispheres was chosen from the anterior margin to the posterior at 4–6 mm to observe the changes in the hippocampus. One slide of the brain tissues was routinely stained with hematoxyline and eosin (H&E) for determining the number of vacuoles, and other slides were reacted with periodic acid Schiff (PAS) for measuring the lipofuscin deposits (*17*, *18*). The numbers of vacuoles and lipofuscin-positive cells were counted in the hippocampal regions. Typical pictures of histology are shown in Figs. 1 and 2. The spongiosis grade was assessed by the following

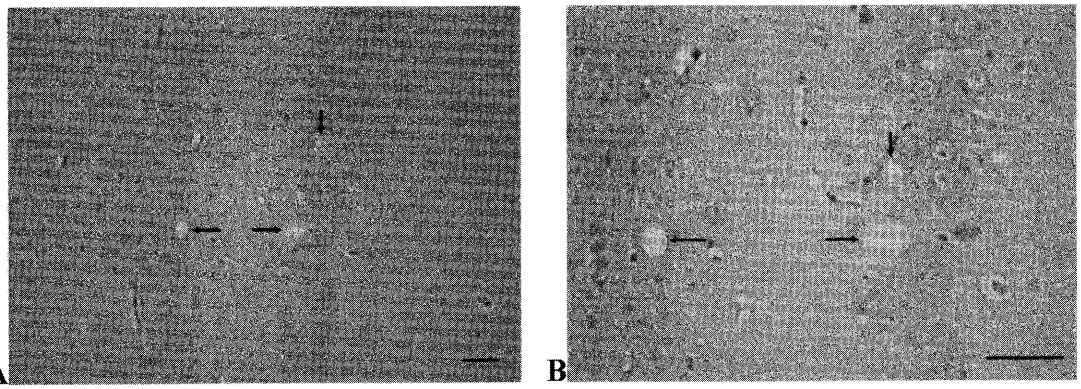

Fig. 1. Vacuoles in the hippocampus of SAMP8 mice. A: numerous vacuoles (arrow) were found in the hippocampus area. B: magnified from A (H&E stain, bar=50 μm).

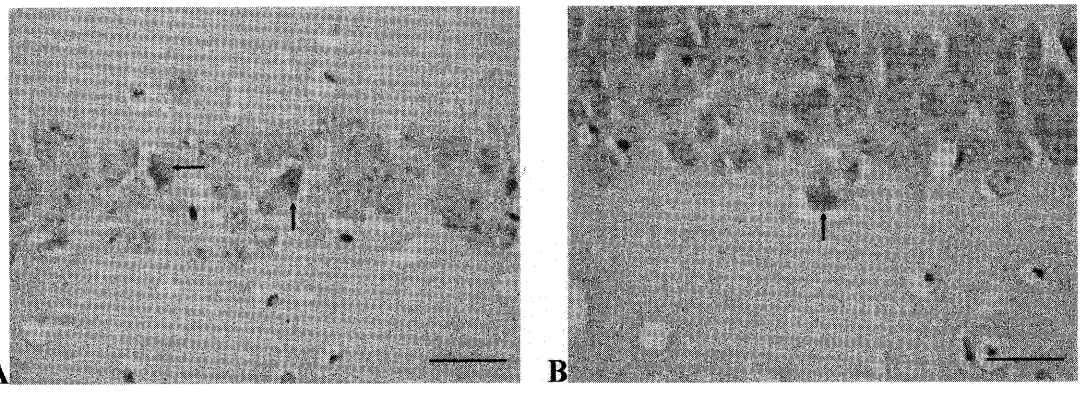

Fig 2. Lipofuscin deposits in the hippocampus of SAMP8 mice. A: two hippocampal pyramidal cells showed normal appearance (arrow). B: lipofuscin deposits in the cytoplasma of pyramidal cell (arrow) (PAS stain, bar=50 μm).

standard: grade 0:<5 vacuoles; grade 1: 5–10 vacuoles; grade 2: 10–30 vacuoles; grade 3: >30 vacuoles, while the percentage of the lipofuscin-positive cells was calculated by the equation: Lipofuscin (%)=number of lipofuscin-positive cells/number of total counted cells× 100% (*19*).

Statistical analyses. All data were expressed as mean± standard error of the mean and analyzed using SPSS 8.0 software (SPSS Inc. Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for the comparison of means between different groups. The least significant difference test was used for pairwise comparisons following a significant ANOVA result. Differences were considered to be significant at $p<0.05$.

RESULTS

Fluid intakes, body weight, and ambulatory activity

The fluid intakes of the oolong tea and green tea groups did not differ in the male groups, whereas the green tea group showed a lower fluid intake than the control and oolong tea groups in female mice ($p<0.05$) (Fig. 3). No differences in body weight or locomotion activity were observed among the control and tea groups ($p>0.05$) (data not shown). Based on the data for fluid intake, the average doses of tea compounds were calculated and are shown in Table 2. The average intakes of gallic acid, epicatechin (EP) and polymeric polyphenols were much higher in the oolong tea group than those in the green tea group in both male and female groups, while the intakes of epigallocatechin gallate (EGCG) and caffeine were much lower in the oolong tea group.

Memory examination

Both the oolong tea and green tea groups showed significantly longer passive avoidance times than those of the control group in both male and female mice, while the passive avoidance time of the oolong tea group was longer than that of the green tea group at the 7th day in the male group ($p<0.05$) (Fig. 4). The mean successful active avoidance times of the oolong and green teas was also higher than those of the control group (Fig. 5), but no difference was found between the oolong and green tea groups. These results indicated that both oolong tea and green tea could lessen the cognitive deficits of mice.

Morphological finding and grading score

The results of the brain morphology changes in the mice are shown in Fig. 6. Both the oolong and green tea groups revealed significantly less spongy degeneration and lower percentages of lipofuscin in the hippocampus than the control group in male mice ($p<0.05$), while no significant difference was observed between the oolong

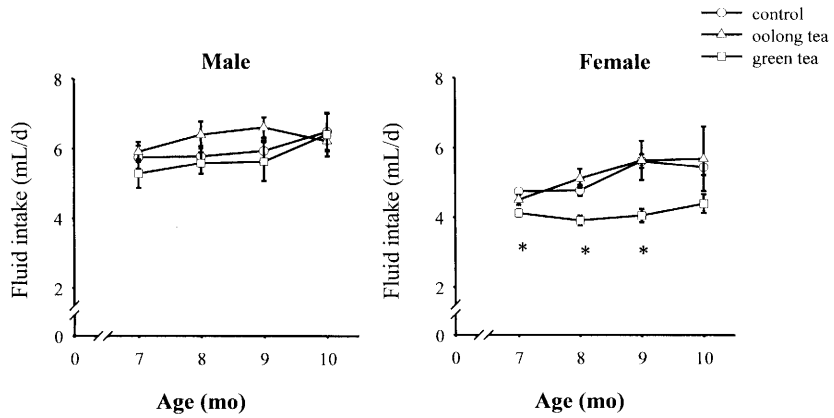

Fig 3. Fluid intakes of SAMP8 mice with different drinks for 16 wk. Values are means±SE. $n=15$ per group. * Data compared with control are significantly different at $p<0.05$. ○, control group; △, oolong tea group; □, green tea group.

Table 2. Daily fluid and corresponding intakes of various compounds for oolong and green tea groups.

	Male		Female	
	Oolong tea	Green tea	Oolong tea	Green tea
Fluid intake (mL/d)	6.3	5.6	5.2	4.1
Gallic acid (μg/d)	140.3	1.7	116.0	1.2
Epigallocatechin (EGC) (μg/d)	1403.2	1510.1	1159.6	1101.7
Catechin (C) (μg/d)	99.4	61.8	82.2	45.1
Epicatechin (EP) (μg/d)	394.5	159.0	326.0	116.0
Epigallocatechin gallate (EGCG) (μg/d)	1446.0	2499.2	1195.0	1823.3
Gallocatechin gallate (GCG) (μg/d)	52.2	36.0	43.2	26.2
Epicatechin gallate (ECG) (μg/d)	292.0	411.4	241.3	300.1
Catechin gallate (CG) (μg/d)	6.3	14.1	5.2	10.3
Polymerized phenols (μg/d)	1223.9	273.7	1011.4	199.7
Caffeine (μg/d)	1389.4	2588.6	1148.2	1888.5

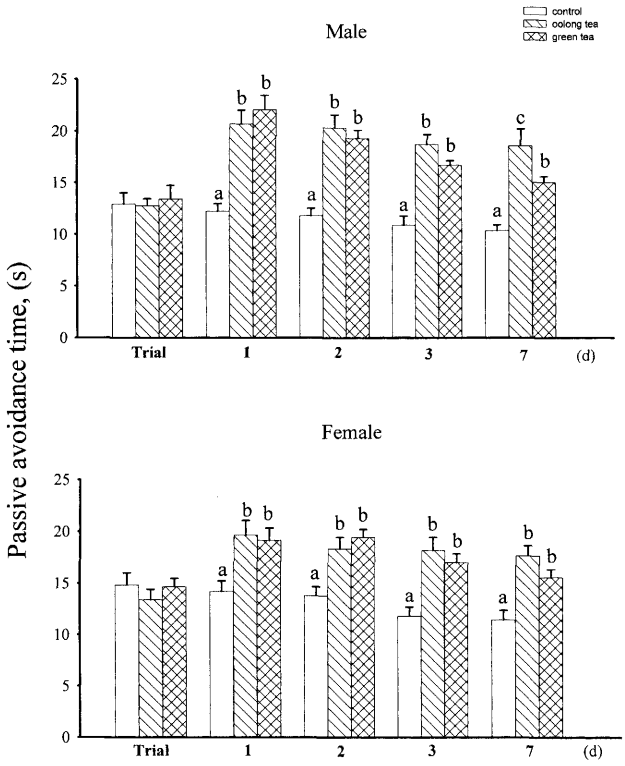

Fig 4. Step-through passive avoidance time of SAMP8 mice with different drinks for 16 wk. Values are means±SE. $n=15$ per group. Data with different superscripts (a>b>c) are significantly different at $p<0.05$.

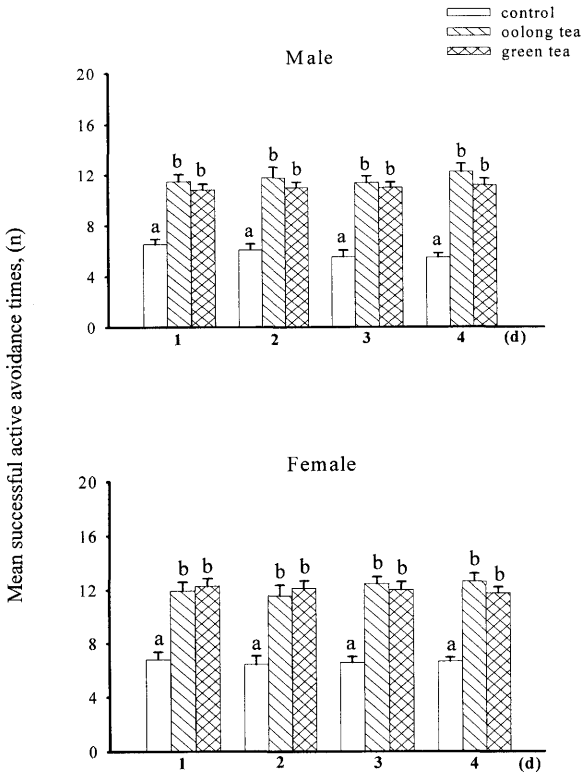

Fig 5. Mean number of successful active (shuttle) avoidance among 20 trials of shuttle avoidance test in male and female SAMP8 mice with different drinks for 16 wk. Values are means±SE. $n=15$ per group. Data with different superscripts (a>b) are significantly different at $p<0.05$.

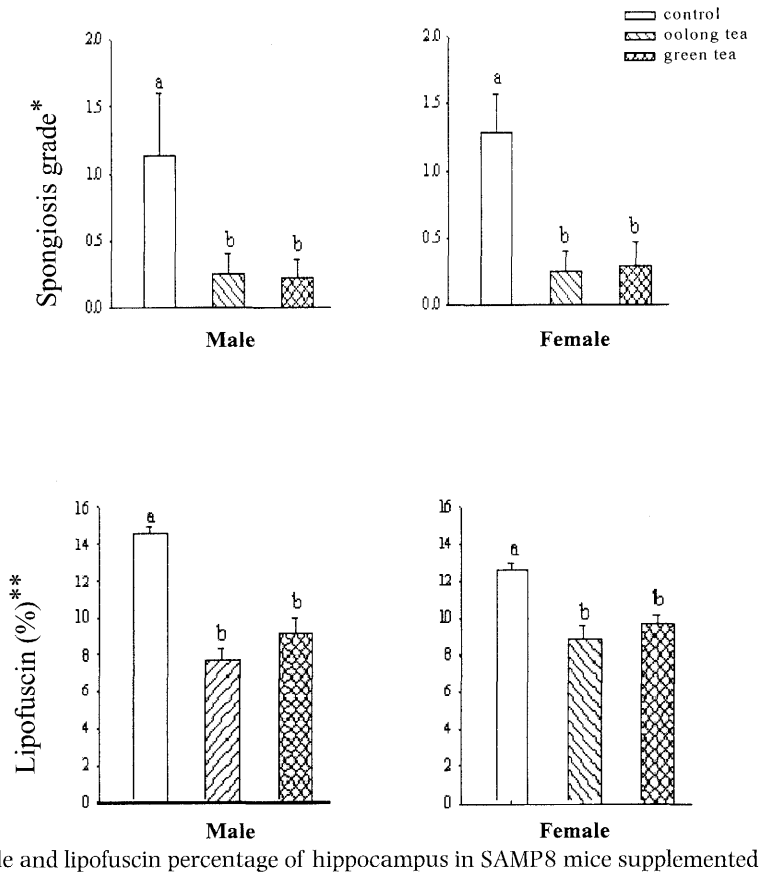

Fig 6. Spongiosis grade and lipofuscin percentage of hippocampus in SAMP8 mice supplemented with different drinks for 16 wk. Values are means±SE. $n=15$ per group. Data with different superscripts (a>b) are significantly different at $p<0.05$. * Assessment standard in 100× photomicroscopy: grade 0: <5 vacuoles; grade 1: 5–10 vacuoles; grade 2: 10–30 vacuoles; grade 3: >30 vacules. ** Lipofuscin (%)=number of positive lipofuscin/number of total nerve cells ×100.

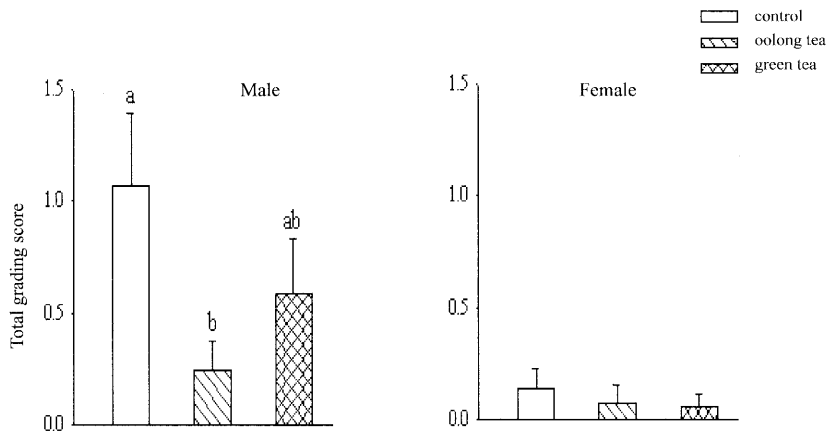

Fig 7. Total grading score of SAMP8 mice supplemented with different drinks for 16 wk. Values are mean±SE. n=15 per group. Data with different superscripts (a>b) are significantly different, at $p<0.05$.

Table 3. Biochemical parameters of SAMP8 mice after supplemented with the water or teas for 16 wk.[1]

Group	Male			Female		
	Control	Oolong tea	Green tea	Control	Oolong tea	Green tea
Total cholesterol (mg/dL)	95.71±2 .28	91.17±2.26	91.67±2.36	70.29±3.12	63.50±2.69	64.14±2.29
Triglyceride (g/dL)	98.29±4.14	84.33±5.21	84.17±5.52	75.43±4.34	67.33±3.91	66.43±3.24
Glucose (mg/dL)	131.43±3.78	122.17±4.78	120.50±4.60	126.43±4.49	120.83±2.61	123.29±2.01
Iron (mg/dL)	246.71±4.99	242.50±2.99	243.33±1.80	245.43±4.31	240.83±2.61	241.29±2.35
Hemoglobin (g/dL)	18.00±1.57	15.20±1.59	16.80±1.32	16.67±1.88	14.00±1.86	15.00±2.08
TEAC[2] in serum	1.20±0.14[a]	1.82±0.27[a b]	1.97±0.23[b]	1.13±0.17[a]	1.87±0.21[b]	1.93±0.22[b]
TEAC[2] in liver	2.98±0.39	3.54±0.27	3.55±0.26	2.33±0.52	3.76±0.81	3.97±0.43

[1] Values are expressed as mean±SE. n=15 per group. Values in the same column not sharing in a common superscript are significantly different from each other at $p<0.05$.
[2] TEAC: Trolox equivalent antioxidant capacity.

and green tea groups. In male groups, the oolong tea group showed a lower total grading score than the control group (Fig. 7), whereas the green tea group tended to be lower without statistical significances ($p>0.05$). It was also noted that the total grading score of the tea groups tended to be lower than that of the control group (Fig. 7) but without statistical significance in female mice ($p>0.05$). These results showed that both oolong tea and green tea have a beneficial effect on slowing the aging process of mice, and had similar results to each other.

Biochemical analyses

The biochemical values of different groups are summarized in Table 3. The concentrations of total cholesterol, triglyceride, glucose, iron and hemoglobin did not differ among three groups in either male or female mice. The serum TEAC level of the male green tea group was significantly higher than that of the male control group, while the oolong tea group tended to be higher than the control group with no statistical significance ($p>0.05$). In female mice, the oolong and green tea groups showed significantly higher serum TEAC levels than the control. It was also noted that both the oolong and green tea groups revealed higher TEAC in the liver than the control group but without statistical significance ($p>0.05$). The results indicated that oolong and green tea might promote the antioxidant ability of mice.

DICUSSION

Antioxidants were reported to play an important role in preventing brain degeneration and might inhibit the age-related deficits in the motor learning and memory ability of rats (*2, 3*). Fukui et al. (*20*) assessed the ability of learning and memory using the water maze and the eight-arm radial maze tasks, and found that vitamin E supplementation to the young significantly accelerated their learning functions before the stress and prevented the deficit of memory caused by the stress. Caffeine was demonstrated to have significant antioxidant abilities in protecting membranes against oxidative damage (*9*), and could reverse the memory disruption induced by intra-nigral MPTP-injection in rats (*21*). Angelucci et al. (*22*) also reported that pre-test caffeine administration at doses of 1 to 30 mg/kg improved the retrieval and consolidation of memory. Our study illustrated that the average caffeine intake of the oolong tea group was lower than that in the green tea (1389.4 μg/d and 2588.6 μg/d in the male group, 1148.2 μg/d and 1888.5 μg/d in the female group, respectively). Compared to the method of Angelucci et al. (*22*), we administered lower caffeine doses than in their study, and both oolong tea and green tea could lower the impair-

ment of cognitive abilities. The polyphenolic compounds of tea could prevent oxidative damage (*23*, *24*), and improve the antioxidant status (*25*), whereas it was noted that most polyphenols might be rapidly conjugated in vivo and lose their antioxidant activity via the blocking of the hydroxyl groups (*26*). Our results also found that both the oolong and green tea groups had higher serum TEAC levels. These results suggested that the administration of oolong or green tea could reduce the cognitive disabilities of SAMP8, possibly through the powerful antioxidative capacity of the teas.

The morphological changes of the hippocampus were evaluated since several cognitive functions rely on the integrity of the hippocampus, and the age-ralated impairments are probably due to the vulnerability of this area. In this study, the lipofuscin accumulation in the hippocampus of both tea groups was significantly lower than those in the control groups ($p<0.05$). Lipofuscin was suggested to correlate with the memory impairment in the brain of aged rats (*27*). Kim et al. (*28*) found that the malondialdehyde (MDA) and carbonyl levels were consistently higher in the hippocampus of SAMP8 than that of senescence resistant-prone mice R1 (SAMR1), indicating that the oxidative damage could be one of the causal factors in the accumulation of lipofuscin. The vacuole numbers, another indicator to evaluate the degrees of pathological degeneration, were reported to be much higher in the old mice than that in the young mice. Our data showed that the oolong and green tea could reduce the development of spongiosis caused by aging. These data suggested that the oolong tea and green tea could lessen the degeneration in the hippocampus of SAMP8 mice, and the possible explanation might be the above-mentioned antioxidant abilities of tea.

Hosokawa et al. (*29*) observed a statistically significant correlation between the total grading score and remaining lifespan. Chen (*30*) evaluated the survival ratio of SAMP8 administered water, oolong tea or green tea solutions, and found the survival ratio decreased in the order: oolong>green>water. When we evaluated the total grading score among the three drink groups, the oolong tea group revealed lower total grading score in male groups, while it did not differ among the three female groups. Our results also showed that the grading score of female mice was lower than that of the male mice, indicating that aging process of females might be slower than that of males of the same age. However, the beneficial effect of tea in retarding the aging processes was not found in female group and needs further study.

Polyphenols, one of the major components of tea, were found to have a strong affinity for protein with high proline content and salivary protein rich-contents. Han et al. (*31*) suggested that drinking tea decreased the iron absorption, and this effect was mainly on nonheme iron, while the absorption of heme iron from cooked meats was not affected by tea consumption. However, Miura et al. (*32*) reported that tea ingestion did not influence plasma cholesterol or triglyceride concentrations in apoprotein E-deficient mice. Our results also illustrated that the consumption of tea did not affect the parameters of nutritional status, such as hemoglobin, iron or cholesterol levels.

The advantage of TEAC assay were the extreme simplicity and rapidity of performance, inclusion of multipoint calibration of the dose-response curve, and suitability for the analysis of large numbers of samples (*15*). Furthermore, this assay need only a small amount of sample and could be automated (*15*). Because of the limitation on blood samples of mice, we chose this assay to measure the total antioxidant capacity of the serum as well as liver samples. Our results showed that both the oolong and green tea groups had significantly higher serum TEAC levels than the control in female groups, while a significant difference was only found between the green tea and control groups in male mice. Von Gadow et al. (*33*) compared the antioxidant activities of green, black and oolong teas by the DPPH radical scavenging method, and found the activity decreased in the order: green>black>oolong. Benzie and Szeto (*34*) showed that green tea has the highest antioxidant power and black tea has the lowest values, while oolong tea demonstrated similar activities to those of black teas, and suggested that the antioxidant power of all teas correlated with the total phenolic content. However, the different intakes of phenol and caffeine in the oolong and green tea groups (Table 2) may be another factor to affect the antioxidant capacity.

In conclusion, our results illustrated that both oolong and green teas could promote the antioxidant ability, lessen and prevent the oxidative damages of brain, and lower the cognitive deficits in SAMP8. The protective components of tea that could slow the aging progression are of interest and worth study.

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