Long-Term Administration of Green Tea Catechins Improves Spatial Cognition Learning Ability in Rats

Abdul M. Haque,* Michio Hashimoto,* Masanori Katakura,* Yoko Tanabe,* Yukihiro Hara,† and Osamu Shido*

*Department of Environmental Physiology, Shimane University Faculty of Medicine, Izumo 693-8501, Japan and †Mitsui Norin Company, Limited, Shinjuku-ku, Tokyo 160-8381, Japan

ABSTRACT Green tea catechins confer potent biological properties including antioxidation and free-radical scavenging. We investigated the effect of long-term oral administration of green tea catechins (PolyphenonE, PE; EGCG 63%; EC 11%; EGC 6%; ECG 6%) mixed with water on the spatial cognition learning ability of young rats. The learning ability of rats administered PE (0%, 0.1%, 0.5%) for 26 wk was assessed in the partially baited 8-arm radial maze. Relative to controls, those administered PE had improved reference and working memory–related learning ability. They also had lower plasma concentrations of lipid peroxides and greater plasma ferric-reducing antioxidation power than controls. Furthermore, rats administered PE had lower hippocampus reactive oxygen species concentrations than controls. We suggest that this improvement in spatial cognitive learning ability is due to the antioxidative activity of green tea catechins.


KEY WORDS: • green tea catechins • memory learning • antioxidants • rats

The free-radical hypothesis suggests that increased production of lipid peroxide (LPO) and reactive oxygen species (ROS), which are produced with free radicals in membrane lipids, causes deterioration of a wide variety of cellular enzymes, subsequently exacerbating the neurodegenerative process (1). Oxidative stress, a condition of cellular prooxidant-antioxidant disturbance in favor of the prooxidant state, also induces the production of ROS, leading to serious functional impairments such as cognitive decline (2). On the other hand, a decrease in hippocampal LPO improves spatial cognition learning ability in aged rats (3), and an increase in antioxidative activity in the hippocampus prevents (4) or ameliorates (5) the impairment of learning ability in rats produced by the infusion of amyloid-β peptide 1–40 into the cerebral ventricle.

Tea is rich in polyphenols contained in the leaves and stems of the tea plant. The main polyphenolic components in green tea are (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), and (-)-epicatechin gallate (ECG) (6). EGCG, the major and most active component of green tea catechins, acts as an antioxidant in the biological system (7) and is rapidly absorbed and distributed mainly into the mucous membranes of the small intestine and the liver; more interestingly, it can cross the blood brain barrier (8). Moreover, oxidative stress–induced neuronal apoptosis is prevented by EGCG treatment of neuronal cells (7). Therefore, in the present study, we investigated, through radial maze tasks, how long-term (26 wk) administration of water containing green tea catechins affected spatial cognition learning ability in rats and the oxidative status of their plasma and brain.

MATERIALS AND METHODS

Animals. All animal experiment protocols were carried out in accordance with the guidelines for animal experimentation of Shimane University compiled from the guidelines for animal experimentation of the Japanese Association for Laboratory Animal Science. Male Wistar rats (n = 24; 5 wk old; Jcl: Wistar; Clea Japan) were randomly divided into 3 groups and orally administered green tea catechins (Polyphenon E, PE; Mitsui Norin) mixed with water, or water alone for 26 wk as follows: a 0.1% group (administered 1 g/L PE; n = 7), a 0.5% group (5 g/L PE; n = 9) and a control group (given water alone; n = 8). The rats were maintained in an air-conditioned animal room with a 12-h dark-light cycle under controlled temperature (23 ± 2°C) and humidity (50 ± 10% relative humidity); the rats had free access to a normal laboratory diet, MF (Oriental Yeast) and tap water with or without PE. The MF diet, a nutritionally adequate and standard solid diet, comprising (in descending order of amount) flour, corn, soybean meal, wheat flour, yeast, alfalfa meal and soybean oil, included the following (g/kg): 70 water, 240 crude protein, 51 crude fat, 62 crude ash, 32 crude fiber, and 545 nitrogen free extract (>90% starch).

Water containing PE es EGCG (63%), EC (11%), EGC (6%), and ECG (6%) was freshly prepared every other day.

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Radial maze–learning ability. Both 2 and 5 mo after starting the PE administration, the rats’ learning ability was tested by an assessment of their behavior in an 8-arm radial maze (Toyo Sangyo) as described (9). Briefly, the rats were trained to acquire a reward (food pellet) at the end of each of the 4 arms of an 8-arm radial maze. The performance involved 2 parameters of memory function: reference memory error (RME), i.e., entry into unbaited arms; and working memory error (WME), i.e., repeated entry into arms that had already been visited in the same trial. Each rat was given 2 trials, 6 d/wk, for a total of 5 wk.

Tissue preparation. After completing the maze task, the rats were anesthetized with sodium pentobarbital (30 mg/kg BW, i.p.), and their blood was collected; the cerebral cortex and hippocampus were then separated as described (4,5). A portion of the frontal cortex (100 mg) was immediately homogenized on ice in 1.0 mL of ice-cold 0.32 mol/L sucrose buffer (pH 7.4) containing 2 mmol/L EDTA, 0.5 mg/ml leupeptin, 0.5 mg/L aprotinin, and 0.2 mmol/L phenylmethylsulfonyl fluoride using a Polytron homogenizer (PCU 2–110; Kinematica). The residual tissues were stored at −80°C after flash-freezing in liquid N2 until use. The homogenates were immediately subjected to the assays described below or stored at −80°C after liquid N2 flash and bath until use.

Measurements of antioxidative status. The LPO concentration was assessed by the TBARS assay of Ohkawa et al. (10), as described (4,5), with the concentration measured in nanomoles malondialdehyde/mg protein. Malondialdehyde levels were calculated relative to a standard preparation of 1,1,3,3-tetraethoxypropane.

Plasma total antioxidant activity was measured by the ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (11) with slight modification. The working FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tripyridyl-s-triazine (TP) in 40 mmol/L HCl and 20 mmol/L FeCl3. 6H2O solution. After mixing 3 mL of the working FRAP reagent with 400 μL plasma or standard solution in a test tube, a second reading was taken at 600 nm. A blank reading with only the FRAP reagent was subtracted from the absorbance of the FRAP reagent with a sample to measure the actual FRAP value of each tube.

The levels of ROS were determined as described (4,5). Briefly, 50 μL of freshly prepared tissue homogenate was mixed with 4.85 mL of 0.1 mol/L potassium phosphate buffer (pH 7.4) and incubated with 2',7'-dichlorofluorescein diacetate (Molecular Probes) in methanol at a final concentration of 5 μmol/L for 15 min at 37°C. The dye-loaded samples were centrifuged at 12,500 × g for 10 min at 4°C. The pellet was mixed on a vortex at 0°C in 5 mL of 0.1 mol/L potassium phosphate buffer (pH 7.4) and incubated for 60 min at 37°C. Fluorescence was measured with a Hitachi 850 spectrophotometer at wavelengths of 488 nm for excitation and 525 nm for emission. The cuvette holder was maintained at 37°C. ROS were quantified from a dichlorofluorescein standard curve in methanol. The protein concentration was estimated by the method of Lowry et al. (12).

Statistical analysis. Results were expressed as means ± SEM. Behavioral data were analyzed by a 2-factor (group and block) randomized block factorial ANOVA; all other variables were analyzed for intergroup differences by 1-way ANOVA. ANOVA was followed by Fisher’s Protected Least Significant Difference test for post hoc comparisons. Correlation was determined by simple regression analysis. The statistical programs used were GB-STAT 6.5.4 (Dynamic Microsystems) and StatView 4.01 (MINDVISION Software, Abacus Concepts). A level of P < 0.05 was considered significant.

RESULTS

PE intake and body weight. Daily water intake did not differ among the control [27.7 ± 1.7 mL/(rat-d)], 0.1% PE [26.0 ± 1.4 mL/(rat-d)], and 0.5% PE [26.2 ± 1.0 mL/(rat-d)] groups. PE intakes were 26.0 ± 1.4 mg/rat-d in the 0.1% PE group and 131 ± 7.0 in the 0.5% PE group. Final body weights did not differ among the groups and were 496 ± 8 g in the control group, 503 ± 10 g in the 0.1% PE group, and 508 ± 11 g in the 0.5% PE group.

Radial-maze learning ability. After 2 mo of PE administration, the scores of RME and WME in block 10 of the radial maze tasks undergone by the 0.5% PE rats were not lower than those of the control and the 0.1% PE rats. Therefore, we reestimated the learning ability (over a period of 6 wk) 20 wk after starting the administration of PE.

The effect of PE administration for 26 wk on reference (Fig. 1A) and working (Fig. 1B) memory-related learning ability is expressed as the mean number of RME and WME for each group, with the data averaged over blocks of 6 trials (Fig. 1). Randomized 2-factor (block and group) ANOVA, for analyzing the effect of PE (0.1 and 0.5%), revealed significant main effects of both blocks of trials (P < 0.0001) and groups (P < 0.0001) on the number of RME (Fig. 1A), but without a significant block × group interaction. Similarly, a significant main effect of both blocks of trials (P < 0.0001) and groups (P = 0.0002) was observed on the number of WME (Fig. 1B), but with a significant block × group interaction (P < 0.0001). Subtest analysis (Table 1) of the number of RME showed the effect of 0.1% PE group on control group (blocks of trials and groups, without a significant block × group interaction); the effect of 0.5% PE group on control groups (blocks of trials and groups, without a significant block × group interaction) and the effect of the PE dose on PE-administered rats (blocks of trials and groups, without a significant block × group interaction), demonstrating that rats administered 0.1 and 0.5% PE had a lower RME score than the control rats (Fig. 1A). Similarly, subtest analysis (Table 1) of the number of WME showed the effect of 0.1% PE group on control group (blocks of trials and groups, with a significant block × group interaction); the effect of 0.5% PE group on control group (blocks of trials and groups, with a significant block × group interaction) and the effect of the PE dose on PE-administered rats (blocks of trials, but not groups), without a significant block × group interaction, demonstrating that rats administered 0.1% and 0.5% PE had a lower WME score than the control rats (Fig. 1B). These analyses suggested that long-term administration of PE improved reference and working memory–related learning ability of rats.

Oxidative status of rat plasma and brains. Plasma TBARS concentrations were dose dependently decreased in the groups administered PE compared with the control group (P = 0.0002, Table 2). The plasma FRAP concentration was higher in the 0.5% PE group than in the control group (P = 0.007) (Table 2). TBARS levels in the hippocampus were reduced in the 0.1 and 0.5% groups, compared with the control group (P = 0.002).
(Table 3). Similarly, the levels of ROS in the hippocampus were reduced in the 0.1 and 0.5% groups, compared with the control group (P = 0.021). Cortex TBARS and ROS levels did not differ among the rat groups. These results indicate that PE has antioxidative effects on oxidative status in rat plasma and the hippocampus.

Regression analysis revealed a significant positive correlation (r = 0.520, P = 0.032) between the hippocampal TBARS levels and the number of RME in block 10 of the radial maze task in control and 0.5% PE-administered rats (Table 4). There was a significant negative correlation between the plasma FRAP levels and the number of RME in block 10 of the radial maze task in control rats and those administered 0.5% PE (r = 0.570, P = 0.017) (Table 4). Similarly, the number of WME in block 10 of the radial maze task in control rats and those administered 0.5% PE correlated positively with plasma TBARS levels (r = 0.622, P = 0.008). The hippocampal TBARS levels and the number of WME tended to be positively correlated (r = 0.480, P = 0.051; Table 4).

**DISCUSSION**

The present study demonstrated that long-term administration of green tea catechines (PE) improves the performance in radial maze tasks and that the level of LPO in the hippocampus correlates significantly with the RME score. Thus, green tea catechines may be involved in protecting against neuronal degenerative stress and in the accumulation of LPO and ROS.

Green tea catechines comprise EGCG, EGC, ECG, and EC and protect the brain, liver, and kidney from lipid peroxidation injury (13). The relative antioxidant activity among tea catechines is EGCG > EGC > ECG > EC (14) Catechins have a protective effect against age-related neurological diseases associated with ROS (15). In this study, long-term (26 wk) administration of PE decreased the plasma and hippocampal oxidative status. In the process of aging, LPO and ROS accumulate and are constantly involved in some of the pathophysiologic effects associated with oxidative stress in cells and tissues. An increase in the production of LPO exacerbates the neurodegenerative process by deteriorating cellular enzymes (1). Antioxidative enzymes are activated by green tea catechin intake (16), and the antioxidative potency of human plasma increases with continual ingestion of green tea (17). These antioxidative defense systems might also prevent oxidative damage in the brain. Long-term intake of green tea catechines may be important because cells are constantly exposed to oxidative stress.

Aging leads to a decline in spatial memory–related learning ability. Oxidative damage to the brain is associated with age-related cognitive dysfunction (18), and some antioxidants are effective in improving such dysfunction; examples include the effects of a garlic extract on aged SAMP10 mice, a model of brain senescence with cerebral atrophy and cognitive dysfunction (19), and of vitamin E on rats with oxidative stress (20). Catechins are more effective radical scavengers than vitamins E and C (21,22). Long-term administration of green tea catechins to SAMP10 mice also suppressed cognitive dysfunction, as demonstrated by the duration of learning needed to acquire an avoidance response and by the assessment of working memory in the Y-maze (23). Chronic administration of catechins for 3.5 mo improved learning memory in maze behavior of both adult and old mice, although the mechanism of the improvement has not been clarified (24). In this study, to estimate the effects of the administration of green tea catechins on the learning ability of rats, PE was administered for 26 wk starting at 5 wk of age. Therefore, the point in time at which the effect of green tea catechins on the improvement of learning ability becomes apparent may differ among animal species.

The hippocampus and the cerebral cortex are the key structures of memory formation. Because the hippocampus is especially indispensable in the integration of spatial information, a decline in learning ability may be induced by the deterioration of hippocampal function. In this study, both a decrease in TBARS levels in the hippocampus and an increase in FRAP levels in the plasma were related to the acquisition of higher reference memory-related learning ability; in addition, a decrease in plasma TBARS levels was related to the acquisition of higher working memory-related learning ability (Table 4). A decrease in hippocampal LPO levels suggests an improvement in spatial cognitive learning memory in aged rats (3). Furthermore, an increase in the antioxidative effects of docosahexaenoic acid on the hippocampus prevents impairment of

**TABLE 2**

Plasma oxidative status of rats administered 0, 0.1% PE, or 0.5% PE for 26 wk

<table>
<thead>
<tr>
<th>Group</th>
<th>TBARS</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>μmol/L</td>
</tr>
<tr>
<td>0%</td>
<td>8</td>
<td>4.02 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1% PE</td>
<td>7</td>
<td>3.51 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5% PE</td>
<td>9</td>
<td>3.00 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are means ± SEM. Means in a column without a common letter differ, P < 0.05.
Oxidative status of cerebral cortex and hippocampus in rats administered 0, 0.1%, or 0.5% PE for 26 wk

<table>
<thead>
<tr>
<th></th>
<th>Cerebral cortex</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBARS</td>
<td>ROS</td>
</tr>
<tr>
<td></td>
<td>n (nmol/mg protein)</td>
<td>pmoI/(min mg protein)</td>
</tr>
<tr>
<td>0%</td>
<td>8 1.452 ± 0.101</td>
<td>0.206 ± 0.027</td>
</tr>
<tr>
<td>0.1% PE</td>
<td>7 1.231 ± 0.102</td>
<td>0.195 ± 0.031</td>
</tr>
<tr>
<td>0.5% PE</td>
<td>9 1.265 ± 0.083</td>
<td>0.186 ± 0.035</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Means in a column without a common letter differ, P < 0.05.

TABLE 4

Correlation coefficients between learning ability and oxidative stress of plasma and hippocampus of rats administered 0, 0.1% PE, or 0.5% PE for 26 wk

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBARS</td>
<td>FRAP</td>
</tr>
<tr>
<td>RME</td>
<td>NS</td>
<td>−0.570</td>
</tr>
<tr>
<td>P-value</td>
<td>−</td>
<td>0.017</td>
</tr>
<tr>
<td>WME</td>
<td>0.622</td>
<td>NS</td>
</tr>
<tr>
<td>P-value</td>
<td>0.008</td>
<td>−</td>
</tr>
</tbody>
</table>

1 The number of RME and WME in block 10 shown in Figure 1 was used as an indicator of learning ability. Differences were considered significant when P < 0.05, NS, not significant, P > 0.05.
CATECHINS IMPROVE SPATIAL COGNITION ABILITY


