Title

Green tea catechins prevent cognitive deficits caused by Abeta1-40 in rats

Author(s)

Abdul M Haque, Michio Hashimoto, Masanori Katakura, Yukihiko Hara, Osamu Shido

Journal

The Journal of nutritional biochemistry, Volume 19, Issue 9

Published

2008 Feb 15

URL

https://doi.org/10.1016/j.jnutbio.2007.08.008

Abdul M. Haque [a], Michio Hashimoto [a*], Masanori Katakura [a], Yukihiko Hara [b], Osamu Shido [a]

[a] Department of Environmental Physiology, Shimane University Faculty of Medicine, Izumo, Shimane 693-8501, Japan

[b] Mitsui Norin Ltd., Shinjuku-ku, Tokyo 160-8381, Japan

Green tea catechins prevent cognitive deficits caused by $A\beta_{1-40}$ in rats

Abdul Haque, Michio Hashimoto*, Masanori Katakura, Yukihiko Hara, Osamu Shido

Faculty of Medicine, Shimane University, Izumo City, Shimane, Japan

Received 8 May 2007; received in revised form 8 August 2007; accepted 16 August 2007

Abstract

Amyloid β peptide (Aβ)-induced oxidative stress is involved in the pathogenesis of Alzheimer's disease (AD). In contrast, green tea catechins confer potent antioxidative defense to brain neurons. Therefore, we examined whether long-term administration of green tea catechins [Polyphenon E (PE): 63% of epigallocatechin-3-gallate, 11% of epicatechin, 6% of (−)-epigallocatechin and 6% of (−)-epicatechin-gallate] prevents cognitive impairment in an animal model of AD rats infused with $A\beta_{1-40}$ into the cerebral ventricle. Five-week-old male Wistar rats fed with an MF diet were randomly divided into two groups: 0.0% PE (rats administered with water only) and 0.5% PE (rats administered with 5 g/L of PE). Twenty weeks after the PE administration, the 0.0% PE group was divided into the Vehicle group (rats infused with the solvent used for dissolving Aβ) and the $A\beta_{1-40}$-infused rat group (Aβ group), whereas the 0.5% PE group was divided into the PE+Vehicle group (PE-preadministered vehicle-infused rats) and the PE+Aβ group (PE-preadministered Aβ-infused rats). $A\beta_{1-40}$ or vehicle was infused into the cerebral ventricle using a mini osmotic pump. Behavioral changes in the rats were assessed by an eight-arm radial maze. PE administration for 26 weeks significantly decreased the Aβ-induced increase in the number of reference and working memory errors, with a concomitant reduction of hippocampal lipid peroxide (LPO; 40%) and cortico-hippocampal reactive oxygen species (ROS; 42% and 50%, respectively). Significantly reduced levels of LPO in the plasma (24%) and hippocampus (25%) as well as those of ROS in the hippocampus (23%) and cortex (41%) were found in the PE+Vehicle group as compared with the Vehicle group. Furthermore, rats with preadministered PE had higher ferric-reducing antioxidation power of plasma as compared with the Vehicle group. Our results suggest that long-term administration of green tea catechins provides effective prophylactic benefits against Aβ-induced cognitive impairment by increasing antioxidative defenses.

Keywords: Green tea catechins; Memory learning; Antioxidants; Alzheimer's disease; Rats

1. Introduction

Amyloid β peptide (Aβ) plays a central role in the etiology of Alzheimer's disease (AD) [1], although it is unclear as to how precisely Aβ contributes to the disease process. Oxidative stress may be involved in the mechanism of Aβ-induced neurotoxicity [2–5] and the pathogenesis of AD [6,7]. For instance, Aβ increases hydrogen peroxide and lipid peroxide (LPO) concentrations in cells [3,8] and membranes [9]. Higher levels of lipid peroxidation [10], protein carbonyl modification [11] and mitochondrial DNA oxidation [12] have also been reported in the brains of AD patients as compared with those of age-matched controls.

We reported that lower hippocampal LPO concentrations attribute to better spatial learning ability in young [13] and aged [14] rats. Consistent with these findings, we further reported that a decrease in hippocampal LPO concentrations and/or an increase in antioxidative defense in the hippocampus prevents [15] and/or ameliorates [16] learning impairment in an animal model of AD rats infused with $A\beta_{1-40}$ into the cerebral ventricle.

Abbreviations: 0.0% PE group, rats administered with water only; 0.5% PE group, rats administered with 5 g/L of PE; Aβ, amyloid β peptide; Aβ group, amyloid β peptide$_{1-40}$-infused rats; AD, Alzheimer's disease; ANOVA, analysis of variance; APP, amyloid precursor protein; EC, epicatechin; ECG, (−)-epicatechin-gallate; EGC, (−)-epigallocatechin; EGCG, epigallocatechin-3-gallate; FRAP, ferric-reducing antioxidation power; LPO, lipid peroxide; LTP, long-term potentiation; PE, Polyphenon E; PE+Aβ group, PE-preadministered Aβ-infused rats; PE+Vehicle group, PE-preadministered vehicle-infused rats; PKC, protein kinase C; PLSD, protected least significant difference; RME, reference memory error; ROS, reactive oxygen species; TBARS, thiobarbituric acid-reactive substances; Vehicle group, rats infused with the solvent used for dissolving Aβ; WME, working memory error.

* Corresponding author.

E-mail address: michio1@med.shimane-u.ac.jp (M. Hashimoto).

Michio Hashimoto
Department of Environmental Physiology, Shimane University Faculty of Medicine,Izumo,Shimane 693-8501, Japan. Phone: +81-853-20-2112, Fax: +81-853-20-2110

Tea is rich in polyphenols contained in the leaves and stems of the tea plant. The major polyphenolic components in green tea are epigallocatechin-3-gallate (EGCG), epicatechin (EC), (−)-epigallocatechin (EGC) and (−)-epicatechin-gallate (ECG). EGCG is the abundant and most active component [17,18] of green tea catechins, acts as an antioxidant in the biological system [19] and attenuates lipid peroxidation caused by various forms of free radicals [20]. In particular, EGCG reduces neuronal cell death caused by transient global ischemia [21], Aβ-induced neurotoxicity [19] and α-amino-3-hydroxy-5-methyl-4-isoxazolo propionate-induced calcium influx and neuronal cell damage [22], all of which are associated with increased oxidative stress. We reported that long-term administration of green tea catechins reduces hippocampal LPO and reactive oxygen species (ROS) levels and increases the ferric-reducing antioxidation power (FRAP) of plasma in rats. These changes demonstrated improved age-related cognitive decline in rats [23]. We therefore investigated whether long-term administration of tea catechins prevents oxidative stress and cognitive impairment in Aβ-infused AD model rats.

2. Materials and methods

2.1. Animals, diet and experimental design

Five-week-old male rats (n=49; Jcl:Wistar, Clea, Osaka, Japan) were housed with a 12-h dark/light cycle under controlled temperature (23±2°C) and humidity (50±10% relative humidity) with ad libitum access to a normal MF diet (Oriental Yeast, Osaka, Japan) and water. The MF diet, which is a nutritionally adequate and standard solid diet for rodents composed of (in descending order of amount) flour, corn, soybean meal, whitefish meal, yea[illegible] soybean oil, included 70 g/kg of water, [illegible] protein, 51 g/kg of crude fat, 62 g/kg of crude ash, 32 g/kg of crude fiber and 545 g/kg of nitrogen-free extract (>90% of which is starch). Rats were randomly divided into two groups and administered with green tea catechins [Polyphenon E (PE), ~~Mitsui Norin, Tokyo Japan~~ Mitsui Norin Ltd., Tokyo, Japan) mixed with water for a total of 26 weeks as follows: the 0.5% (w/v) PE group (rats administered with 5 g/L of PE; n=24) and the 0.0% PE group (rats administered with water only; n=25). The water containing PE as EGCG (63%), (−)-EC (11%), EGC (6%) and ECG (6%) was freshly prepared every other day. The experimental design details are diagrammed in Fig. 1. We followed the general guidelines for the care and use of laboratory animals as recommended by the Shimane University and compiled from the guidelines for animal experimentation of the Japanese Association for Laboratory Animal Science.

2.2. Infusion of $A\beta_{1-40}$ into rats

The infusion of $A\beta_{1-40}$ (Peptide Institute, Osaka Japan) into the cerebral ventricle was essentially the same as described previously [15]. Briefly, rats were anesthetized lightly with sodium pentobarbital (50 mg/kg ip). The skull was exposed and two holes (right and left, relative to the bregma; 0.8 mm posterior and 1.4 mm lateral) were drilled according to the atlas of Paxinos and Watson [24] using a stereotaxic frame (Narishige, Tokyo, Japan). The $A\beta_{1-40}$ was injected into the left cerebral ventricle using a mini osmotic pump. Rats other than those from the $A\beta_{1-40}$-infused group (Aβ group) were administered with the [illegible] dissolving Aβ) only. The outlet of [illegible]d 3.5 mm into the left ventricle. The infusion rate was 0.5 μl/h, and the total amount of infused Aβ was 4.9–5.5 nmol. We injected 0.5 μg of $AlCl_3$ into the right cerebral ventricle before implanting the mini osmotic pump to facilitate the aggregation of $A\beta_{1-40}$.

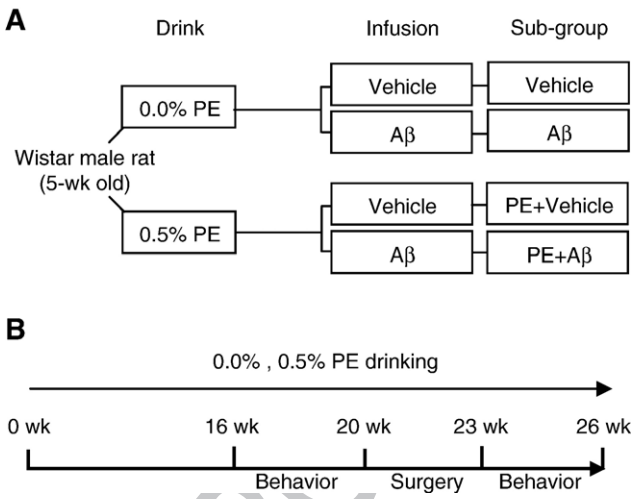

Fig. 1. Experimental design: study grouping (A) and schedule (B). Five-week-old male Wistar rats were fed with 0.0% PE or 0.5% PE for a total of 26 weeks. At that time, rats were behaviorally tested for uniform subgrouping with an eight-arm radial maze. A vehicle or $A\beta_{1-40}$ was infused into the cerebral ventricle of the rats from the 0.0% PE and 0.5% PE groups, which were subsequently subdivided into the Vehicle, Aβ, PE +Vehicle and PE+Aβ groups. Finally, rats were behaviorally tested to assess the effects of PE on cognitive learning ability.

2.3. Behavioral assessment by radial maze

Rats were behaviorally tested to study their learning-related cognitive ability with the use of an eight-arm radial maze (Toyo Sangyo, Toyama, Japan) as described previously [13,14]. Briefly (Fig. 1), 16 weeks after the PE administration, rats in the two groups (0.0% PE group and 0.5% PE group) were tested to perform a standard task in an eight-arm radial maze. Before the preliminary behavior test began, rats were transferred to a regimen of food deprivation to keep their body weight at 80–85% of their free feeding weight, and each rat was handled for 5 min everyday for a total of 5 consecutive days with constant monitoring of body weight. The rats were then familiarized with the radial maze apparatus, across the entire surface of which reward pellets were scattered. After the end of the 1-week adaptation period, each rat was given two daily trials for 3 weeks in which the reward acquisition at

the end of each arm was recorded. After they completed this behavior test, each group of rats was subdivided into two uniform groups allowing for the number of errors made by each rat in the last six trials in the preliminary behavior test and infused with Aβ or the vehicle as follows: the 0.0% PE group was divided into rats infused with the solvent used for dissolving Aβ (Vehicle group, n=12) and an Aβ-infused group (Aβ group, n=13), whereas the 0.5% PE group was divided into a vehicle-infused PE group (PE+Vehicle group, n=12) and an Aβ-infused PE group (PE+Aβ group, n=12). The four groups of rats were behaviorally tested at 3 weeks after surgery to assess the effect of PE preadministration on cognitive learning ability. This testing lasted for a total of 3 weeks. The same protocol used for the preliminary behavior test was followed in the final behavior test except for the adaptation period. The performance involved two parameters of memory function: reference memory error (RME, entry into unbaited arms within a trial) and working memory error (WME, repeated entry into any arm that had already been visited within a trial). Lower numbers of RMEs and WMEs implied better spatial learning ability of the rats.

2.4. Plasma and brain collection

After their completion of the behavioral test, the rats were anesthetized with sodium pentobarbital (65 mg/kg ip); blood samples were collected, and brains were quickly isolated as described previously [15]. The tissues were prepared for biochemical analyses as described previously [23] and stored at −80°C until analysis.

2.5. Determination of plasma lipid

Plasma triglyceride and total cholesterol levels were enzymatically measured with a Triglyceride E-Test and a Cholesterol E-Test (Wako Pure Chemical, Osaka, Japan), respectively.

2.6. Determination of oxidative status

LPO concentrations were determined by the thiobarbituric acid-reactive substances (TBARS) assay as described previously [25]. Briefly, the reaction mixture, containing 50 μl of homogenates, 100 μl of 8.1% sodium dodecyl sulfate and 1.5 ml of a 0.8% solution of thiobarbituric acid in a 20% acetic acid solution (pH 3.5), was made up to a final volume of 2.0 ml with distilled water. The mixture was heated at 95°C for 60 min. After cooling the mixture with tap water, we added 500 μl of distilled water and a 2.5-ml mixture of n-butanol and pyridine (15:1, v/v). Then, the whole mixture was shaken vigorously for 15 min. After centrifugation at 2500×g for 20 min, the absorbance of the organic (upper) layer was measured at 532 nm. TBARS levels are expressed as nanomoles of malondialdehyde per milligram of protein. Malondialdehyde concentrations were calculated relative to a standard preparation of 1,1,3,3-tetraethoxypropane. Protein concentrations were determined according to the method of Lowry et al. [26].

The concentrations of ROS were determined as described previously [15,27]. Briefly, freshly prepared tissue homogenate was mixed with 100 mmol/L of potassium phosphate buffer (pH 7.4) and incubated with 2′,7′-dichlorofluorescein diacetate 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 4°C in 100 mmol/L of phosphate buffer (pH 7.4) and incubated again for 60 min at 37°C. Fluorescence intensity was measured with a spectrofluorometer (Type 850, Hitachi, Tokyo, Japan) at wavelengths of 488 nm for excitation and 525 nm for emission. The cuvette holder was maintained at 37°C. The ROS concentrations were quantified from a dichlorofluorescein standard curve in methanol.

Plasma total antioxidant activity was measured by the assay of FRAP with slight modification [28]. Briefly, the working reagent of FRAP was prepared by mixing 300 mmol/L of acetate buffer (pH 3.6) and 10 mmol/L of 2,4,6-tripyridyl-s-triazine in a solution of 40 mmol/L of HCl and 20 mmol/L of $FeCl_3 \cdot 6H_2O$. Absorbance was taken at 600 nm after mixing the working FRAP reagent with plasma or standard solution. A blank reading with only the FRAP working reagent was subtracted from the absorbance of the FRAP reagent with a sample to measure the actual FRAP value of each tube.

2.7. Statistical analyses

Values are expressed as mean±S.E.M. Statistical analyses of the data were carried out using the GB-STAT 6.5.4 (Dynamic Microsystems, Silver Spring, MD, USA) and StatView 4.01 (MindVision Software, Abacus Concepts, Berkeley, CA, USA) programs. Behavioral data were tested by two-way (group and block) randomized block factorial analysis of variance (ANOVA). Intergroup differences of all other parameters were analyzed by one-way ANOVA followed by Fisher's protected least significant difference (PLSD) test with post hoc comparisons. Correlation was determined by measuring Pearson's product–moment correlation coefficient, referred to as r.

3. Results

3.1. PE intake and body weight

mg/Kg body weight.

Rats in all groups did not differ in daily intake volume of water or PE-mixed water. The daily intake of PE was 304±7 ~~mg/kg~~. The final body weight did not differ among the groups (Vehicle group, 471±9 g; Aβ group, 464±7 g; PE+Aβ group, 465±9 g; PE+Vehicle group, 478±7 g).

3.2. Effects of PE preadministration on radial maze learning ability

The effect of PE preadministration on reference and working memory-related learning ability in the vehicle and $A\beta_{1-40}$-infused AD model rats is expressed as the mean number of RMEs and WMEs for each group, with the data

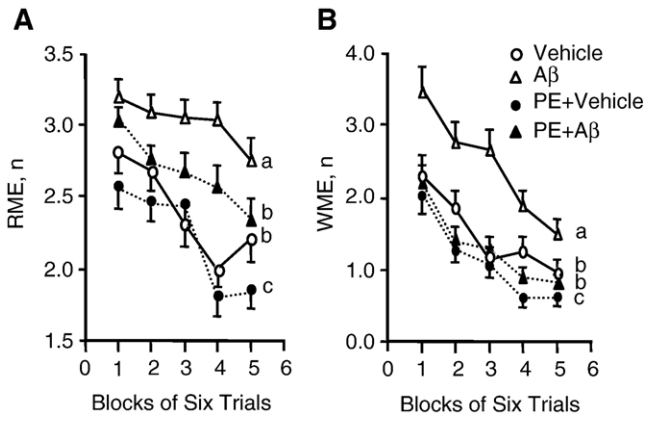

Fig. 2. Effects of PE preadministration to $A\beta_{1-40}$-infused rats on reference and working memory-related learning ability in the eight-arm radial maze task. Each value represents the number of RMEs (A) and that of WMEs (B) as the mean±S.E.M. in each block of six trials. Groups without a common letter for the main effects are significantly different at $P<.05$: Vehicle group, $n=12$; Aβ group, $n=13$; PE+Vehicle group, $n=12$; PE+Aβ group, $n=12$. The significance of differences among the four groups was determined by randomized two-factor (block and group) ANOVA followed by Fisher's PLSD test. Main effects of blocks of trials and groups were significant ($P<.0001$) on the number of RMEs and that of WMEs but without a significant Block×Group interaction. Details of the subtest analysis between the two groups of main effects of blocks of trials and groups are shown in Table 1.

averaged over blocks of six trials (Fig. 2). Randomized two-factor (block and group) ANOVA revealed significant main effects of blocks of trials [$F(4,308)=14.66$, $P<.0001$] and groups [$F(3,231)=26.87$, $P<.0001$] on the number of RMEs and that of WMEs [blocks: $F(4,308)=41.23$, $P<.0001$; groups: $F(3,231)=32.13$, $P<.0001$] but without a significant Block×Group interaction on the number of RMEs ($P=.141$) and that of WMEs ($P=.582$) (Fig. 2). Subset analyses (Table 1) of the number of RMEs showed the effect of PE on the Vehicle and Aβ-infused groups and the effect of Aβ on the Vehicle and PE+Vehicle groups, demonstrating that the PE+Aβ and PE+Vehicle groups had lower RME scores as compared with the Aβ and Vehicle groups, respectively (Fig. 2). Similarly, subset analyses (Table 1) of the number of WMEs showed the effect of PE on the Vehicle and Aβ-infused groups and the effect of Aβ on the Vehicle and PE+Vehicle groups, demonstrating that the PE+Aβ and PE+Vehicle groups had lower WME scores as compared with the Aβ and Vehicle groups, respectively (Fig. 2).

3.3. Effect of PE preadministration on plasma triglyceride and total cholesterol levels

There was no significant difference in the content of plasma triglycerides among the experimental groups (data not shown). However, the total cholesterol content in plasma was significantly lower in the PE+Aβ group (39.51±3.8 mg/dl) than in the Aβ (62.33±2.4 mg/dl), Vehicle (69.89±3.9 mg/dl) and PE+Vehicle (61.50±3.4 mg/dl) groups ($P<.05$).

3.4. Effect of PE preadministration on the oxidative status of plasma and brain

Plasma TBARS concentrations were significantly lower in the PE+Vehicle group as compared with the Vehicle, Aβ and PE+Aβ groups [$F(3,45)=3.58$, $P=.020$; Table 2]. On the other hand, the plasma FRAP concentrations were significantly higher in the PE-preadministered groups (PE+Vehicle and PE+Aβ groups) as compared with the water-administered groups (Vehicle and Aβ groups) [$F(3,45)=11.87$, $P<.0001$; Table 2).

The hippocampal TBARS [$F(3,45)=16.88$, $P<.0001$] and ROS [$F(3,45)=16.23$, $P<.0001$] concentrations were significantly higher in the Aβ group than in the Vehicle, PE+Vehicle and PE+Aβ groups (Table 2). However, Aβ rats with preadministered PE (PE+Aβ group) had significantly lower TBARS [$F(1,23)=24.80$, $P<.0001$] and ROS [$F(1,23)=26.50$, $P<.0001$] concentrations as compared with the Aβ group (Table 2). Significantly lower TBARS [$F(1,22)=8.48$, $P=.0081$] and ROS [$F(1,22)=11.21$, $P=.0029$] concentrations were also found in the PE-preadministered group (PE+Vehicle) as compared with the Vehicle group (Table 2).

In the cerebral cortex, the TBARS concentrations were unaffected among the four groups [$F(3,45)=1.61$, $P=.200$; Table 2]. The infusion of Aβ displayed significantly higher ROS concentrations in the Aβ group as compared with the Vehicle group [$F(1,23)=4.48$, $P=.0461$]; however, PE preadministration suppressed these effects to the levels of the Vehicle group (Table 2). The PE+Vehicle group had significantly lower ROS concentrations as compared with the Vehicle group [$F(1,22)=5.34$, $P=.039$; Table 2].

t1.1 Table 1
t1.2 Results of the two-factor ANOVA and PLSD test conducted on RME and WME data obtained from the Vehicle ($n=12$), Aβ ($n=13$), PE+Aβ ($n=12$) and PE+Vehicle ($n=12$) groups *

t1.3		RME		WME	
t1.4		Block	Group	Block	Group
t1.5	Vehicle vs. PE+Vehicle	<0.0001 [$F(4,284)=12.72$]	0.0182 [$F(1,71)=5.84$]	<0.0001 [$F(4,284)=15.47$]	0.0023 [$F(1,71)=9.98$]
t1.6	Aβ vs. Vehicle	<0.0001 [$F(4,308)=6.37$]	<0.0001 [$F(1,77)=35.45$]	<0.0001 [$F(4,308)=17.31$]	<0.0001 [$F(1,77)=24.19$]
t1.7	Aβ vs. PE+Aβ	0.0009 [$F(4,308)=4.78$]	<0.0001 [$F(1,77)=22.76$]	<0.0001 [$F(4,308)=19.73$]	<0.0001 [$F(1,77)=53.28$]
t1.8	Aβ vs. PE+Vehicle	<0.0001 [$F(4,308)=7.43$]	<0.0001 [$F(1,77)=65.63$]	<0.0001 [$F(4,308)=20.29$]	<0.0001 [$F(1,77)=79.66$]
t1.9	Vehicle vs. PE+Aβ	<0.0001 [$F(4,284)=7.14$]	0.0286 [$F(1,71)=4.99$]	<0.0001 [$F(4,284)=16.22$]	0.1811 [$F(1,71)=1.82$]

t1.10 * Data are presented in Fig. 2.

t2.1 Table 2
t2.2 Oxidative status of plasma, cerebral cortex and hippocampus of the Vehicle, Aβ, PE+Vehicle and PE+Aβ rats *

t2.3		Vehicle (n=12)	Aβ (n=13)	PE+Aβ (n=12)	PE+Vehicle (n=12)
t2.4	Plasma				
t2.5	TBARS (nmol/ml)	3.90±0.26[a]	3.95±0.27[a]	3.73±0.25[a]	2.97±0.28[b]
t2.6	FRAP (μmol/L)	116.6±8.9[b]	126.5±5.4[b]	165.5±9.8[a]	171.5±7.3[a]
t2.7	Cortex				
t2.8	TBARS (nmol/mg protein)	3.05±0.26	3.13±0.23	2.74±0.22	2.50±0.21
t2.9	ROS (pmol/mg protein/min)	0.153±0.027[b]	0.223±0.020[a]	0.130±0.018[b,c]	0.090±0.017[c]
t2.10	Hippocampus				
t2.11	TBARS (nmol/mg protein)	2.52±0.11[b]	3.54±0.05[a]	2.10±0.13[b,c]	1.89±0.19[c]
t2.12	ROS (pmol/mg protein/min)	0.222±0.015[b]	0.322±0.027[a]	0.162±0.014[c]	0.171±0.013[b,c]

t2.13 * Values are expressed as mean±S.E.M. Mean values in a row with superscript letters without common letters differ, P<.05.

3.5. Correlations between learning ability and TBARS and ROS concentrations in plasma and brain

Regression analysis revealed significant positive correlations between the number of RMEs and the concentrations of TBARS in plasma (r=0.324, P=.023; Fig. 3A) and the hippocampus (r=0.44, P=.016; Fig. 3B). A similar correlation was found between the hippocampal ROS concentrations and the number of WMEs (r=0.294, P=.041; Fig. 3C). On the other hand, a negative correlation was observed between the FRAP concentrations and the number of WMEs (r=−0.296, P=.039; Fig. 3D). A statistically nonsignificant but high tendency of a positive correlation between the hippocampal ROS concentrations and the number of RMEs (r=0.280, P=.051, Fig. 3E) and a tendency of a negative correlation between the FRAP concentrations and the number of RMEs (r=−0.272, P=.058; Fig. 3F) were observed in the final block of the radial maze test.

4. Discussion

The present study demonstrates that long-term preadministration of PE markedly prevents $A\beta_{1-40}$-induced spatial cognitive learning impairment in AD model rats. PE preadministration consistently suppressed Aβ-induced increases in LPO and ROS concentrations in the brain and plasma, suggesting that the antioxidative action of PE could be involved in preventing cognitive impairment in Aβ-infused rats.

The free radical hypothesis of AD suggests that increased production of LPO changes a wide variety of cellular enzymes and exacerbates the neurodegeneration processes [29]. The hippocampus and cerebral cortex are key components for memory formation, and the hippocampus is uniquely indispensable in the integration of spatial information. In this study, we found that PE preadministration significantly suppressed Aβ-induced LPO and ROS production in the brain and concomitantly improved memory-related learning ability. We assume that neuroprotection might play a role in the favorable effect of PE against Aβ-induced oxidative insults and cognitive deficits. This is because vitamin E and ferulic acids demonstrate similar effects in learning and memory deficiencies in Aβ-infused rats [30] and mice [31], respectively. We thus speculate that lower LPO and ROS concentrations, combined with the higher acquisition of memory performance, are likely to be the effects of PE on scavenging and/or preventing radical formation at the neuronal level.

PE is composed of EGCG, EGC, ECG and EC. The relative antioxidant potential of tea catechins is EGCG=ECG>EGC>EC [20]. Metabolism of green tea catechins has been studied in animal [32] and human [33] subjects. After oral administration, EGCG is detected as free EGCG, its conjugates or both and peaks at 1–2 h after dose administration in rat systemic circulation [34]. Studies with radioactively labeled EGCG in mice [35] or chemiluminescence-based detection in rats [36] also demonstrated its incorporation into the brain and into other organs, such as the kidney, heart, liver, spleen and pancreas.

Long-term potentiation (LTP) is a form of synaptic plasticity widely studied as a cellular basis for learning and memory formation [37]. Aβ infusion into the rat hippocampus evidently induces a deficit in LTP and working memory [38]. Age-related impairment is also linked to age-related increases in hippocampal ROS concentrations [39]. Here, Aβ infusion significantly increased the hippocampal ROS concentrations and impaired the learning-related cognitive functions (Fig. 2; Table 2). In addition, PE preadministration increased the FRAP concentrations (an indicator of the total antioxidant potential of plasma), which negatively correlated with the number of RMEs and that of WMEs. Thus, with all the evidence taken together, we speculate that the improvement of learning ability after long-term PE preadministration is due to either changes in the antioxidant and/or radical scavenger concentrations or an increase in the antioxidizing activities and consequent prevention of Aβ-induced LTP impairment in AD rats. This speculation is consistent with findings that oral administration of tea catechins activates the antioxidative enzymes in mice [40] and that supplementation with antioxidant-rich diets reverses the age-related LTP deficits by increasing antioxidative defenses in rats [41,42].

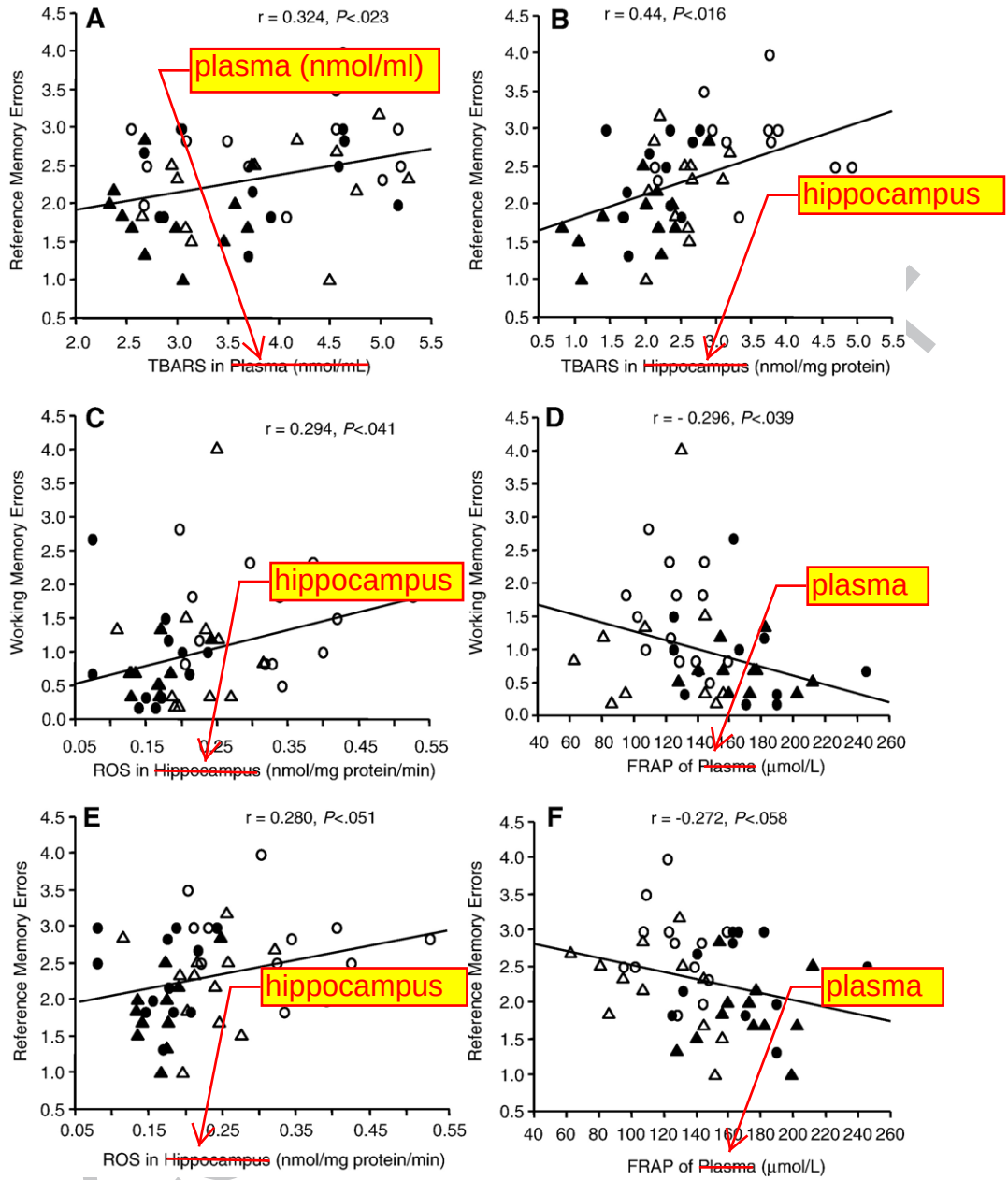

Fig. 3. Scatter plots of the relationship between learning ability and each of the TBARS, ROS and FRAP levels. Learning ability is expressed as the number of RMEs and that of WMEs in Block 5. ○, Aβ group (n=13); △, Vehicle group (n=12); ▲, PE+Vehicle group (n=12); ●, PE+Aβ group (n=12).

reference memory errors and that of working memory errors

Other than its antioxidant and radical-scavenging actions, EGCG modulates the production of Aβ by regulating its synthesizing enzymes [43]. Administration of EGCG for 4–7 days significantly increases the expression of protein kinase C (PKC)-α and PKCε, the two specific [illegible] protein (APP) processing in human SH-SY5Y neuroblastoma cells and in mice [43]. In addition, EGCG administration markedly increases the α-secretase cleavage activity, decreases $A\beta_{1-40,42}$ levels and attenuates Aβ plaques across the hippocampal and cortical brain regions in $TgAPP_{sw}$ mice, a mouse model of AD [44]. Furthermore, epidemiological and experimental data demonstrate that hypercholesterolemia is an early risk factor for the development of the amyloid pathology of AD [45,46]. In the present study, the total cholesterol content in plasma was significantly decreased in the PE+Aβ group, but the mechanism is not [illegible] evated level of cholesterol accelerates Aβ production in AD by shifting APP metabolism from the α- to the β-cleavage pathway [47], and lowering the cholesterol by simvastatin reduces the production of Aβ in vitro and in vivo [48]. Therefore, green tea catechins may have another effective role to prevent cerebral amyloidosis in AD by modulating cholesterol metabolism.

The process of aging increases oxidative stress and induces the production of ROS, leading to serious functional impairments, including cognitive decline [49]. Cells are constantly exposed to oxidative stress, and brain tissues are especially vulnerable due to their inherently poor antioxidative defense mechanisms. EGCG has a stronger antioxidant activity as compared with either vitamin E or C on a molar basis in vitro [50]. Furthermore, in reducing ferrous ion-induced lipid peroxidation, the IC_{50} values of several antioxidants are as follows: 3.32 μM for EGCG, 75.65 μM for trolox, 7.63 μM for lipoic acids and 15.48 μM for melatonin [51]. In addition, higher consumption of green tea is associated with lower prevalence of cognitive impairment in elderly people [52]. Therefore, as compared with other antioxidants, long-term consumption of green tea catechins might have a higher preventive effect on cognitive deficits. In this study, the intake volume of PE-mixed water was approximately 60 ml/kg/day in the 0.5% PE group. Based on this water volume intake, a person (with a body weight of 50 kg) would have to drink about 3 L of PE per day to get similar effects. However, humans consume antioxidants (including vitamins A, B, C and E as well as polyphenols, etc.) from various food sources everyday. Therefore, a lower amount (<3 L) of 0.5% PE-mixed water volume intake may be effective in humans to ensure the similar effects. However, detailed investigation is certainly required to understand the fate of catechins in humans.

In conclusion, our results suggest that long-term administration of PE prevents cognitive deficits caused by oxidative stress, Aβ induced and/or otherwise, at least by facilitating antioxidative defenses. However, further research is required to clarify the exact mechanism of how PE contributes to the prevention of cognitive deficit in AD model rats.

References

[1] Selkoe DJ. Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. Ann NY Acad Sci 2000; 924:17–25.

[2] Behl C, Davis J, Cole GM, Schubert D. Vitamin E protects nerve cells from amyloid β protein toxicity. Biochem Biophys Res Commun 1992;186:944–52.

[3] Behl C, Davis JB, Lesley R, Schubert D. Hydrogen peroxide mediates amyloid beta protein toxicity. Cell 1994;77:817–27.

[4] Butterfield DA, Hensley K, Harris M, Mattson M, Carney J. Aβ-amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: implications to Alzheimer's disease. Biochem Biophys Res Commun 1994;200:710–5.

[5] Schubert D, Behl C, Lesley R, Brack A, Dargusch R, Sagara Y, Kimura H. Amyloid peptides are toxic via a common oxidative mechanism. Proc Natl Acad Sci U S A 1995;92:1989–93.

[6] Yankner BA. Mechanisms of neuronal degeneration in Alzheimer's disease. Neuron 1996;16:921–32.

[7] Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med 1997;23:134–47.

[8] Harris ME, Hensley K, Butterfield DA, Leedle RA, Carney JM. Direct evidence of oxidative injury produced by the Alzheimer's β-amyloid peptide (1–40) in cultured hippocampal neurons. Exp Neurol 1995; 131:193–202.

[9] Mark RJ, Fuson KS, May PC. Characterization of 8-epiprostaglandin $F_{2\alpha}$ as a marker of amyloid β-peptide-induced oxidative damage. J Neurochem 1999;72:1146–53.

[10] Subbarao KV, Richardson JS, Ang LC. Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro. J Neurochem 1990;55:342–55.

[11] Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR. Excess brain protein oxidation and enzyme dysfunction in normal aging and Alzheimer disease. Proc Natl Acad Sci U S A 1991;88:10540–3.

[12] Mecocci P, MacGarvey U, Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann Neurol 1994;36: 747–51.

[13] Gamoh S, Hashimoto M, Sugioka K, Hossain MS, Hata N, Misawa Y, Masumura S. Chronic administration of docosahexaenoic acid improves reference memory-related learning ability in young rats. Neuroscience 1999;93:237–41.

[14] Gamoh S, Hashimoto M, Hossain S, Masumura S. Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats. Clin Exp Pharmacol Physiol 2001;28: 266–70.

[15] Hashimoto M, Hossain S, Shimada T, Sugioka K, Yamasaki H, Fujii Y, et al. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. J Neurochem 2002; 81:1084–91.

[16] Hashimoto M, Tanabe Y, Fujii Y, Kikuta T, Shibata H, Shido O. Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid β-infused rats. J Nutr 2005;135:549–55.

[17] Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radic Biol Med 1996;21:895–902.

[18] Nanjo F, Mori M, Goto K, Hara Y. Radical scavenging activity of tea catechins and their related compounds. Biosci Biotechnol Biochem 1999;63:1621–3.

[19] Choi YT, Jung CH, Lee SR, Bae JH, Baek WK, Suh MH, et al. The green tea polyphenol (−)-epigallocatechin gallate attenuates β-amyloid-induced neurotoxicity in cultured hippocampal neurons. Life Sci 2001;70:603–14.

[20] Gau Q, Zhao B, Li M, Shen S, Xin W. Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. Biochim Biophys Acta 1996;1304: 210–22.

[21] Lee S, Suh S, Kim S. Protective effects of the green tea polyphenol (−) epigallocatechin gallate against hippocampal neuronal damage after transient ischemia in gerbils. Neurosci Lett 2000;287:191–4.

[22] Bae JM, Mun KC, Park WK, Lee SR, Suh SI, Baek WK, et al. EGCG attenuates AMPA-induced intracellular calcium increase in hippocampal neurons. Biochem Biophys Res Commun 2002;290:1506–12.

[23] Haque AM, Hashimoto M, Katakura M, Tanabe Y, Hara Y, Shido O. Long-term administration of green tea catechins improves spatial cognition learning ability in rats. J Nutr 2006;136:1043–7.

[24] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.

[25] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.

[26] Lowry OH, Rosebrough N, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193: 265–75.

[27] Hashimoto M, Hossain MS, Shimada T, Yamasaki H, Fujii Y, Shido O. Effects of docosahexaenoic acid on annular lipid fluidity of the rat bile canalicular plasma membrane. J Lipid Res 2001;42:1160–8.

[28] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239:70–6.

[29] Yatin SM, Aksenov M, Butterfield DA. The antioxidant vitamin E modulates amyloid β-peptide-induced creatine kinase activity inhibition and increased protein oxidation: implications for the free radical hypothesis of Alzheimer's disease. Neurochem Res 1999;24:427–35.
[30] Yamada K, Tanaka T, Han D, Senzaki K, Kameyama T, Nabeshima T. Protective effects of idebenone and α-tocopherol on β-amyloid-(1–42)-induced learning and memory deficits in rats: implication of oxidative stress in β-amyloid-induced neurotoxicity in vivo. Eur J Neurosci 1999;11:83–90.
[31] Yan JJ, Cho JY, Kim HS, Kim KL, Jung JS, Huh SO, et al. Protection against β-amyloid peptide toxicity in vivo with long-term administration of ferulic acid. Br J Pharmacol 2001;133:89–96.
[32] Kohri T, Matsumoto N, Yamakawa M, Suzuki M, Nanjo F, Hara Y, et al. Metabolic fate of (−)-[4-^{3}H] epigallocatechin gallate in rats after oral administration. J Agric Food Chem 2001;49:4102–12.
[33] Lee MJ, Wang ZY, Li H, Chen L, Sun Y, Gobbo S, et al. Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol Biomarkers Prev 1995;4:393–9.
[34] Unno T, Takeo T. Absorption of (−)-epigallocatechin gallate into the circulation system of rats. Biosci Biotechnol Biochem 1995;59:1558–9.
[35] Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, Fujiki H. Wide distribution of [^{3}H](−)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. Carcinogenesis 1998;19:1771–6.
[36] Nakagawa K, Miyazawa T. Absorption and distribution of tea catechin, (−)-epigallocatechin-3-gallate, in the rat. J Nutr Sci Vitaminol 1997;43:679–84.
[37] Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993;361:31–9.
[38] Stephan A, Laroche S, Davis S. Generation of aggregated β-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. J Neurosci 2001;21:5703–14.
[39] O'Donnell E, Vereker E, Lynch MA. Age-related impairment in LTP is accompanied by enhanced activity of stress-activated protein kinases: analysis of underlying mechanisms. Eur J Neurosci 2000;12:345–52.
[40] Khan SG, Katiyar SK, Agarwal R, Mukhtar H. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. Cancer Res 1992;52:4050–2.
[41] McGahon BM, Martin DS, Horrobin DF, Lynch MA. Age-related changes in LTP and antioxidant defenses are reversed by an α-lipoic acid-enriched diet. Neurobiol Aging 1999;20:655–64.
[42] Murray CA, Lynch MA. Dietary supplementation with vitamin E reverses the age-related deficit in long-term potentiation in dentate gyrus. J Biol Chem 1998;273:12161–8.
[43] Levites Y, Mandel AT, Youdim MB. Neuroprotection and neurorescue against Aβ toxicity and PKC-dependent release of non-amyloidogenic soluble precursor protein by green tea polyphenol (−)-epigallocatechin-3-gallate. FASEB J 2003;17:952–4.
[44] Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeanniton D, et al. Green tea epigallocatechin-3-gallate(EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci 2005;25:8807–14.
[45] Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. Arch Neurol 2000;57:1439–43.
[46] Refolo LM, Malester B, La Francois J, Bryant-Thomas T, Wang R, Tint GS, et al. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. Neurobiol Dis 2000;7:321–31.
[47] Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K. Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. Proc Natl Acad Sci U S A 1998;95:6460–4.
[48] Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, et al. Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. Proc Natl Acad Sci U S A 2001;98(8):5856–61.
[49] Liu R, Liu IY, Bi X, Thompson RF, Doctrow SR, Malfroy B, et al. Reversal of age-related learning deficits and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. Proc Natl Acad Sci U S A 2003;100:8526–31.
[50] Rice-Evans CA. Implications of the mechanisms of action of tea polyphenols as antioxidants in vitro for chemoprevention in humans. Proc Soc Exp Biol Med 1999;220:262–6.
[51] Lee SR, Im KJ, Suh SI, Jung JG. Protective effect of green tea polyphenol (−)-epigallocatechin gallate and other antioxidants on lipid peroxidation in gerbil brain homogenates. Phytother Res 2003;17:206–9.
[52] Kuriyama S, Hozawa A, Ohmori K, Shimazu T, Matsui T, Ebihara S, et al. Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project 1. Am J Clin Nutr 2006;83:355–61.