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Long-term administration of green tea catechins increases antioxidative actions and enhances neurogenesis in the hippocampus of rats

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Running title: Neuroprotection effect of catechins

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Abstract

We reported that green tea catechins prevent oxidative stress and improve spatial cognition learning ability in rats. To understand the mechanisms, we investigated the effects of green tea catechins on mRNA expression and activity of antioxidative enzymes and the effect on hippocampal neurogenesis in rat brain. Green tea catechins [Polyphenon E (PE): 63% (-)-epigallocatechin gallate, 11% (-)-epicatechins, 6% (-)-epigallocatechin and 6% (-)-epicatechin gallate] was administered to male Wistar rats. After 26 weeks of PE administration rat brains were isolated and measured the mRNA levels and activity of antioxidative enzymes. To investigate the effect of PE on neurogenesis, 5-bromo-2'-deoxy uridine (BrdU) was injected for 5 consecutive days and stained for BrdU positive cells. PE administration increased the enzyme activity and mRNA levels of catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase in the cerebral cortex and/or hippocampus compared to controls. PE administration also increased number of BrdU-Neuronal nuclei double positive cells in the dentate gyrus of hippocampus than controls. These results indicate that long-term administration of green tea catechins increases antioxidative action and neurogenesis in brain. This is possibly related to the mechanisms involved in improving cognitive function of green tea catechins-administered rats.

1. Introduction

Green tea is rich in polyphenolic compounds known as catechins. The major polyphenolic components of green tea catechins are (-)-epicatechins (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG). Among them, EGCG is the most active component and acts as an antioxidant in the biological system. Moreover, it can cross the blood-brain barrier (Nakagawa et al., 1997) and prevents oxidative stress-induced neuronal apoptosis (Choi et al., 2001) and ischemic-induced neurodegenaration (Sutherland et al., 2005).

In the process of aging, oxidative stress is increased and it induces a disorder of cellular functions (Harman 1981). Oxidative damage to neuronal biomolecules such as DNA, proteins and lipids contributes to the pathogenesis of neurodegenerative diseases including age-related cognitive decline (Halliwell et al., 1989; Willis et al., 2009). The brain is especially vulnerable to oxidative damage due to its relatively lower antioxidant capacity compared to other organs, high consumption of oxygen, and high content of oxidizable polyunsaturated fatty acids (Butterfield et al., 2007). Therefore, a balance between the cellular oxidative stress and the antioxidative defenses in the brain is particularly important.

We reported that long-term administration of green tea catechins improves spatial cognition learning ability in normal rats (Haque et al., 2006), and prevents impairment of learning ability in amyloid-β peptide$_{1\text{-}40}$ (A$\beta_{1\text{-}40}$)-infused Alzheimer's disease (AD) model rats (Haque et al., 2008). Unno et al. (2007) also showed that consumption of green tea catechins delays the memory deficit in senescence-accelerated (SAM P10) mice, a model

for examining brain aging and antisenescence effects. In addition to animal studies, epidemiological studies demonstrated that higher consumption of green tea is associated with lower prevalence of cognitive impairment in elderly people (Kuriyama et al., 2006; Ng et al., 2008). Although the role of green tea catechins in cognitive function is beginning to emerge, very little is known about how it specifically contributes to the improvement of cognitive functions.

An increase in antioxidative defence in the hippocampus prevents (Hashimoto et al., 2002) and ameliorates (Hashimoto et al., 2005) the impairments of cognitive function in $A\beta_{1\text{-}40}$-infused AD model rats. Oxidative stress-induced neuronal damage is counterbalanced by the antioxidant defense system. In particular, the concentration of reactive oxygen species (ROS) in brain is controlled by endogenous antioxidant enzymes such as, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and nonenzymatic scavengers like reduced glutathione. In addition to antioxidative mechanism, on hippocampal neurogenesis plays an important role in learning memory (Leuner et al., 2006). The dentate gyrus (DG) of the hippocampus is one of the regions where neurons are generated throughout the lifespan (Dupret et al., 2008). The newly born cells express neuronal markers, emit axons and receive synaptic inputs and exhibit electrophysiological properties very similar to those of mature dentate granule neurons (Leuner et al., 2006). In this study, we investigated whether long-term administration of green tea catechins (Polyphenon E, PE) affects the gene expression and activities of antioxidative enzymes in brain. The effects of PE administration on hippocampal neurogenesis and neuronal cell survival were also examined.

2. Methods and materials

2.1. Animals and diet

The experiment was performed in strict compliance with the *guidelines for animal experimentation of Shimane University* compiled from *the guidelines for animal experimentation of the Japanese Association for Laboratory of Animal Science*. Rats were house individually in plastic cages in a room equipped with automatic light cycles (light, 7:00-19:00; dark, 19:00-7:00), maintained at 23 ± 2^{o}C and a relative humidity of 50 ± 10%. The rats had free access to diet, (MF; Oriental Yeast, Osaka, Japan) and water. The MF diet is nutritionally adequate and a standard solid diet, comprising of flour, corn, soybean meal, whitefish meal, yeast, alfalfa meal and soybean oil, which 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 green tea catechins [Polyphenon E (PE), Mitsui Norin Co Ltd, Tokyo, Japan] as EGCG (63%), EC (11%), EGC (6%) and ECG (6%) was freshly prepared every other day, given to treated group animals in the drinking water and at the same time water left in the bottle was measured to determine amount consumed.

2.2. Experimental grouping of rats

Male Wistar rats (n=32; 5 wk old; *Jcl*: Wistar; Clea, Japan) were used in this experiment. A detailed experimental design is diagrammed in **Fig 1.** In brief, rats were initially divided into two groups: control (n=16; administered water only) and 0.5% PE group (n=16; administered green tea catechins; PE, 5 g/L). After 26 weeks of PE

administration, 6 rats from both the control and 0.5% PE groups were used for enzyme activity and mRNA expression analyses. The remaining 20 rats (n=10 from control group; n=10 from 0.5% PE group) were used to demonstrated hippocampal neurogenesis as follows: 5 rats of control and 5 rats of 0.5% PE were injected with 5-bromo-2'-deoxy uridine (BrdU; 50 mg/kg/day; i.p.) dissolved in PBS (-) for 5 consecutive days before sacrificing the rats. The other rats (control, n=5; 0.5% PE, n=5) were injected with BrdU (50 mg/kg/day; i.p. 5 consecutive days) five weeks before sacrificing the rats. To clarify the role of PE on neurogenesis, the neuronal precursor proliferation and newborn cell fate (survival) were evaluated by counting BrdU and NeuN (Neuronal Nuclei) double positive cells in the DG at the different time points (1 day and 5 weeks) after last BrdU injection.

2.3. Tissue preparation for enzyme activity assays

Rats used for gene expression and enzyme activity analyses (control; n=6 and 0.5% PE; n=6) were anesthetized using sodium pentobarbital (50 mg/kg B.W., i.p.). Then the brains were immediately removed (within 5 minutes), washed repeatedly in ice-cold saline, and dissected on ice-cold glass dishes into the following regions: cerebral cortex and hippocampus as previously described (Hashimoto et al., 2005). The cerebral cortex and hippocampus were immediately frozen with liquid nitrogen and stored in -80^{o}C until analysis. The brains were homogenized with ice-cold 0.32 mol/L sucrose buffer (pH 7.4) containing 2 mmol/L EDTA, 0.5 mg/L leupeptin, 0.5 mg/L pepstatin, 0.5 mg/L aportinin and 0.2 mmol/L phenayl methylsulfonyl fluoride using a polytron homogenizer (PCU 2-110; Kinematica, Littau-Lucerne, Switzerland) as described (Hashimoto et al., 2002). After centrifuging the ice-cold homogenates at 10,000 x *g* for 5 min at 4^{o}C, the resultant

supernatant was used for determining enzyme activities as described below.

2.4. Superoxide dismutase activity assay

A commercially available superoxide dismutase assay kit (SOD assay kit-WST, Dojindo. Tokyo, Japan) was used to selectively determine the total SOD and MnSOD activities. The activity of SOD was expressed as U/mg protein (one unit is the amount that reduced absorbance by 50% at 450nm). Results were normalized on the basis of total protein content (U/mg protein). MnSOD activity was measured by the addition of 1mmol/L KCN to inhibit the activity of Cu-ZnSOD. The protein concentration was estimated by the method of Lowry et al., (1951).

2.5. Catalase activity assay

The CAT activity was assayed as described (Johansson et al., 1988; Wheeler et al., 1990). Briefly, the reaction mixture contained 0.21 mol/L phosphate buffer (pH 7.0), 0.5 mol/L methanol, 0.84 mmol/L phosphate buffer (pH 7.8), and 0.74 mmol/L hydrogen peroxide. Diluted samples were mixed and then shaken for 20 min at 20^{o} C. The reaction was terminated by the addition of 0.43 mol/L potassium hydroxide, followed by the rapid addition of 11.5 mmol/L 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole. After mixing, the mixtures were incubated for 20 min at 30^{o}C. Then 5.49 mmol/L potassium periodate in 0.5 mol/L potassium hydroxide was added. The absorbance of the purple formaldehyde adduct was measured at 550 nm. The CAT activity was determined by linear least-squares regression of the absorbance of formaldehyde standards. The activity of CAT was expressed as mU/mg protein (one unit is the amount of enzyme that utilizes 1 μmol of

hydrogen peroxide/min).

2.6. Glutathione peroxidase activity assay

The activity of GPx was determined with minor modifications of method [20]. Briefly, the reaction mixture contained 0.05 mol/L phosphate buffer (pH 7.0) with 5 mmol/L EDTA, 8.4 mmol/L NADPH, 1U GR, 12.5 mol/L sodium azide, 0.15 mol/L reduced glutathione and 2.2 mmol/L hydrogen peroxide. Active site of GPx was reduced by incubation for 3.5 min at 20^{o}C. We then added sample and continuously recorded absorbance for the maximum rate of reaction at 340 nm for 5 min using a Hitachi U-3210 spectrophotometer. The activity of GPx was expressed as mU/mg protein (one unit is the amount of enzyme that utilizes 1 μmol of NADPH oxidized /min).

2.7. Glutathione reductase activity assay

The GR activity was measured as described (Carlberg et al., 1985). The reaction mixture contained samples of 0.2 mol/L phosphate buffer (pH 7.0) with 2 mmol/L EDTA, 2 mmol/L NADPH in 10 mmol/L tris-HCl (pH 7.0) and 20 mmol/L oxidized glutathione. The reaction mixture was mixed and the decreased absorbance was spectrophotometrically measured at 340 nm for 10 min at 25^{o}C. A unit of GR activity is defined as the amount of enzyme that catalyzes the reduction of 1 μmol of NADPH/min. Specific activity is expressed as mU/mg protein.

2.8. Total RNA extraction and cDNA synthesis for gene expression analyses

Total RNA was isolated from the hippocampus and cerebral cortex using the Bio-Rad

Aqua Pure RNA isolation kit (Bio-Rad, CA, USA), and treated with RNase-free DNase to remove any residual genomic DNA as recommended by the manufacturer. cDNAs were synthesized incubating total RNA (1 μg), superscript II RNase H-reverse transcriptase (200 U), oligo-$(dT)_{12-18}$ primer (100 nmol/L), dNTPs (1mmol/L) and RNase-inhibitor (40 U) for 60 min at 42°C. The reaction was terminated by incubating for 10 min at 70°C.

2.9. Real-Time quantitative PCR

Specific primers to amplify CAT, GPx, GR, and MnSOD are listed in **Table 1**. Each PCR reaction contained 0.2 μmol/L each primers, 1 x Quantitect SYBR Green PCR master mix, and template cDNA. PCR amplifications were performed with the ABI Prism 7000 Sequence Detection System (Applied Biosystems, CA, USA). The PCR condition for CAT and GPx was as follows: activation of enzymes (15 min at 95°C); denaturation, annealing and amplification (40 cycles: 15 sec at 95°C, 30 sec at 58°C and 30 sec at 72°C respectively). For GR and SOD, we used the following cycles: activation of enzyme (15 min at 95°C); denaturation, annealing and amplification (40 cycles: 15 sec at 95°C, 30 sec at 60°C and 30 sec at 72°C respectively). Real-time detection of fluorimetric intensity of SYBR green I, indicating the amount of PCR product, was measured at the middle point of the linier elongation phase. A melting curve analysis was performed to check for specificity of PCR reaction. The expression of each target gene was normalized with the mRNA expression levels of GAPDH. For each sample, CAT, GPx, GR and MnSOD mRNA expression levels were calculated by means of the comparative cycle threshold (Ct) method using $2^{-\Delta\Delta Ct}$ according to the manufacturer's instruction as described in the Applied Biosystems users bulletin. The fold change in target gene relative to the endogenous control

was determined using the following equation:

Fold change = $2^{-\Delta\Delta Ct}$

$-\Delta\Delta Ct = (Ct_{target} - Ct_{GAPDH})_{0.5\% PE} - (Ct_{target} - Ct_{GAPDH})_{Control}$

The control group was defined as the calibrator in this experiment. Therefore, the amounts of mRNA in samples were assigned numbers relative to the levels in the calibrator sample.

2.10. Tissue preparation and immunohistochemistry

Five weeks and 1 day after the last BrdU injection, the rats were deeply anesthetized with sodium pentobarbital (i.p., 50 mg/kg B.W.) and perfused transcardially with PBS (-) solution and fixed with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2). Then, the brains were removed, post-fixed for 24 h in 4% paraformaldehyde and replaced by 25% sucrose solution. The brains were sliced coronally in 40 μm sections on a microtome and subjected to immunohistochemical analysis. Immunocytochemical detection of BrdU-labeled nuclei was described previously (Kawakita et al., 2006). Briefly, free-floating brain sections were pretreated with 50% formamide -2×SSC (0.3 mol/L sodium chloride and 0.03 mol/L sodium citrate) at 65°C for 2 h and then incubated with 2 mol/L hydrochloride at 37°C for 30 min. The sections were treated with 0.1 mol/L borate buffer (pH 8.5) at 25°C for 10 min and then incubated with blocking solution containing 3% goat serum and 0.25% Triton X-100 in 0.1 mol/L Tris-HCl buffer (TBS) (pH 7.5) at room temperature for 60 min. Finally, sections were incubated overnight in rat anti-BrdU antibody (1:25) and mouse anti-NeuN antibody (1:500) in TBS containing 1% goat serum

and 0.25% Triton X-100 at 4°C. After being washed with TBS, Alexa 633-conjugated goat anti rat IgG secondary antibody (1:200) and Alexa 488 conjugated goat anti mouse secondary antibody (1:500) were added and incubated at room temperature for 60 min in a dark place. To estimate the number of neurons, the brain sections were washed with TBS and then we mounted with coverslips on glass slides. Finally, fluorescent signals of Alexa 633 and Alexa 488 were visualized under a confocal laser scanning microscope (Olympus Fluoview, Tokyo, Japan).

2.11. Stereology

The number of immunoreactive nuclei of BrdU-NeuN double positive cells was counted in the granule cell layer. Every sixth section (240 μm apart) was selected throughout the rostrocaudal extent of the granuale cell layer in the complete dentate gyrus. Each microscopic image was digitized. BrdU immunoreactive nuclei were counted on a computer monitor to improve visualization and in one focal plane to avoid over sampling. The reference volume of the granuale cell layer was determined by summing the granule cell areas for each section and multiplying by the distance between sections sampled. Total BrdU and NeuN double positive cells were expressed as the mean ± SEM for each hemisphere.

2.12. Statistical analysis

Data are expressed as mean ± SEM. The comparison of the 0.5% PE group with the control group was statistically analyzed by *t*-test. $P<0.05$ was considered statistically

significant.

3. Results

3.1. PE Intake and body weight

The water volume intake did not differ between the control (27.7 ± 1.6 mL/rat/day) and 0.5% PE group (26.2 ± 1.5 mL/rat/day). Based on the water volume intake, the amount of PE intake was approximately 131 ± 7.0 mg/rat/day in the 0.5% PE group. There were no significant differences in body weights between the experimental groups (480 ± 13 g in the control group, 460 ± 13 g in the 0.5 % PE group).

3.2. Effects of PE administration on antioxidant enzymes activity in the cerebral cortex and hippocampus

The enzyme activities of CAT, GPx, GR and SOD were significantly increased in 0.5% PE-rats both in the cerebral cortex and hippocampus compared with those of the control group ($P<0.05$). The activities of CAT, GPx, GR, total SOD and MnSOD were 1.3, 1.4, 1.9, 2.1, 3.1 fold higher in cerebral cortex and 1.3, 1.2, 1.5, 2.1, 2.1 fold higher in hippocampus respectively, of the 0.5% PE-administered rats, when compared to the control group (**Fig. 2 and Fig. 3**).

3.3. Effects of PE administration on antioxidative enzyme mRNA expression levels in cerebral cortex and hippocampus

To investigate the effects of PE administration on gene expression of antioxidative

enzymes in brain, the mRNA levels of CAT, GPx, GR and MnSOD were measured by real time PCR. In the hippocampus, the mRNA levels of CAT, GPx, GR and MnSOD were significantly increased in the 0.5% PE group (3, 1.2, 5 and 6.6 fold, respectively) compared with those of the control group ($p<0.05$; **Table 2**). However, in the cerebral cortex, the mRNA levels were unaffected between the control and 0.5% PE group (Table 2).

3.4. Effects of PE administration on neurogenesis in the hippocampus

Using a confocal microscope, BrdU-incorporated cells were found throughout the dentate gyrus with normal granule cell morphology, and appeared ovoid or round with uniform BrdU staining throughout the nucleus. Furthermore most BrdU(+) cells also expressed to NeuN in the control and PE administered groups, indicating that most of the BrdU incorporated cells differentiated into mature neurons in the dentate gyrus (**Fig. 4**). The number and phenotype of the BrdU positive cells were determined at one day after (Fig. 4A) and at five week after (Fig. 4B) the last BrdU injections. Rats with pre-administered 0.5% PE had significantly higher BrdU-NeuN double positive cells compared to the controls. At one day after the last BrdU injection, the number of double immunoreactive BrdU-NeuN cells in the entire granule layer of dentate gyrus were significantly increased in the 0.5% PE group (Fig. 4D**,** Left panel; control: 342.36 ± 60; 0.5 % PE: 568.51 ± 62, $P<0.05$). Similarly, five weeks after the last BrdU injection, the number of BrdU-NeuN double positive neurons were significantly higher in the 0.5% PE group compared with the control group (Fig. 4D**,** Right panel; control: 267 ± 180; 0.5% PE: 393 ± 30, $P<0.05$). This difference was not due to the difference in the size of the granule cell layer of dentate gyrus. In particular, the reference volume of the analyzed area did not differ between the control

and the 0.5% PE administered groups (control: 1.058 ± 0.0515 mm^3; 0.5% PE: 1.059 ± 0.0392 mm^3, P=0.957).

4. Discussion

We previously reported that administration of green tea catechins decreases brain LPO and ROS levels, and concomitantly improves spatial cognition learning ability in rats (Haque et al., 2006). In the present study, long-term PE administration increases the activity and mRNA expression of antioxidant enzymes in the brain. In addition, rats with pre-administered PE showed higher numbers of adult-born neurons in dentate gyrus of the hippocampus, suggesting that green tea-induced spatial memory improvement observed in our previous studies (Haque et al., 2006; 2008) might be, in part, related to the increase of antioxidative action and/or hippocampal neurogenesis in rat brains.

In process of aging, the antioxidant defense system is impaired in different brain regions such as the cerebral cortex, hippocampus, striatum and cerebellum (Siqueira et al., 2005). The hippocampus and cerebral cortex are the key structures of memory formation. The hippocampus is especially indispensable for spatial memory formation. In the present study, we found that long-term PE administration increased the enzyme activities of CAT, GPx, GR and MnSOD in the cerebral cortex and hippocampus in rats (Fig. 2 and Fig. 3). These findings are consistent with a previous study (Kishido et al., 2007), in that consumption of green tea catechins prevents a decline in GPx activity and protein oxidative damage in aging mouse brains. Administration of a catechin-containing antioxidant preparation also demonstrates an increase in SOD activity in the mitochondrial fraction of

the striatum and midbrain, a decreased LPO formation in the cortex and cerebellum of aged rats (Komatsu et al., 2000). Therefore, it is suggesting that PE administration increases antioxidative action in the brain, at least in part, by enhancing antioxidative enzyme activities. These changes might be involved in preventing age-related oxidative damage and cognitive decline, since a decrease in the lipid peroxide (LPO) level has been shown to improve spatial cognition learning ability in rats (Gamoh et al., 1999; 2001).

Among various isoforms of SOD, the mitochondrial manganese-dependent superoxide dismutase (MnSOD) contributes to a major role in the detoxification of superoxide radicals and prevention of neuronal cell death (Keller et al., 1998; Gonzalez-Zulueta et al., 1998). The efficacy of SOD as an antioxidant depends on its cooperative action with other enzymes like CAT and GPx. CAT detoxifies high concentrations of H_2O_2 (Baud et al., 2004; Kono et al., 1982) although its activity is much lower in the brain. GPx and GR are also important in regulating the level of H_2O_2 (de Haan et al., 1998; Dringen et al., 2005). GPx limits the production of LPO by catalyzing the conversion of peroxidated lipid directly into lipid alcohol (McCray et al., 1976). On the other hand, GR indirectly facilitates to deactivate oxygen radicals (Huang et al., 1996). A decrease in GR activity increases GSSG accumulation, which in turn reduces the intracellular GSH pool and eventually predisposes the neuronal cells towards oxidative damage in the brain. In this study, PE administration increases mRNA expression of CAT, GPx, GR and MnSOD only in the hippocampus (Table 2), but not in the cerebral cortex. It is difficult to explain why gene expression of these antioxidative enzymes was affected only in the hippocampus of 0.5% PE-rats. Possibly the fact that the control levels of CAT

and SOD activities are 2-3 fold higher in the hippocampus compared to the cerebral cortex may affect some of the differences in mRNA expression in these regions. Translational and post-translational regulation of these enzymes is also an important determinant to change the activity in response to oxidative stress (Ho et al., 1996). Chronic administration of green tea catechins reduces the levels of LPO and reactive oxygen species (ROS) in the hippocampus, but not in the cerebral cortex in rats (Haque et al., 2006). It is thus likely that green tea catechins exert a stronger antioxidative effect in the hippocampus than in the cerebral cortex to protect oxidative damage. Taken together, it is suggested that long-term administration of green tea catechins might induce an adaptive change in antioxidative defense mechanism in the brain, particularly in the hippocampus.

Hippocampal neurogenesis participates in the processing and storage of new information (Gould et al., 1999). Although the functional role of neurogenesis remains an open question, evidence suggests that survival of new neurons in DG may specifically be linked with cognitive function (Wati et al., 2006). A positive correlation between neurogenesis and learning improvement has been shown in senescent rats (Drapeau et al., 2003). In contrast, interruption of hippocampal input/output pathways and/or damage closely related structures contributes to memory deficits (Jarrard et al., 1984). In this study, the results provide the first demonstration that long-term administration of green tea catechins increases neuronal precursor proliferation and newborn neuronal survival in the DG of hippocampus, based on the numbers of double positive BrdU-NeuN cells measured at 1 day and 5 weeks after the last BrdU injection (Fig 4). One day and 5 weeks after the last BrdU injection seemed to be suitable times to study cell proliferation and survival,

respectively. Because, the cell cycle of hippocampal progenitor cells is believed to last about 25 hours (Cameron et al., 2001) and, therefore, this may be regarded as a sufficient time to allow cells labeled in the first period of S phase (cell proliferation) to divide. Similarly, the loss of a consistent part of labeled cells was shown to occur within 2 weeks after the last cell division and afterward, their number stabilized (Cameron et al., 2001; Gould et al., 1999). Therefore, staining of BrdU-NeuN double positive cells at 5 weeks after last BrdU injection seems an adequate time to correctly evaluate newborn neuronal cell survival.

Neuronal cells are constantly exposed to oxidative stress. EGCG has a stronger antioxidant activity as compared with either vitamin E or C on a molar basis in vitro (Rice-Evans 1999). Furthermore, in reducing ferrous ion-induced lipid peroxidation, the IC_{50} values of several antioxidants are as follows: 3.32 μmol/L for EGCG, 75.65 μmol/L for trolox, 7.63 μmol/L for lipoic acids and 15.48 μmol/L for melatonin (Lee et al., 2003). Therefore, as an antioxidant, PE may reduce the apoptosis process. It may also contribute to the inhibition or neutralization of free radicals that in turn might modulate or activate the expression of genes, which influence neurogenesis. EGCG prevents oxidative stress-induced neuronal cell damage via activation of PKC and modulation of the expression of several cell survival/cell-cycle genes such as Bax, Bad, Mdm2, Bcl-2, Bcl-w, and Bcl-x(L) in vitro (Mandel et al., 2004; Li et al., 2009). In addition, EGCG induces rapid translocation of PKCα to the membrane compartment (Reznichenko et al., 2005), which is particularly important in neuronal growth and differentiation in the brain. Moreover, EGCG stimulates phosphorylation of the cyclic AMP-response element binding protein resulting

in increases in the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus of mice (Levites et al., 2002). This neurotrophic factor plays an important role in neuronal differentiation and viability. However, to understand precise mechanisms of green tea catechins in fostering neurogenesis and survival of neurons, further investigation is warranted. In this study, the rats were given green tea catechins at a dose of 0.5% PE for 26 weeks. This dosage regimen is well-tolerated by animals and has been shown to be effective in preventing oxidative stress and memory improvement in rats (Haque et al., 2006; 2008). Based on the daily water volume intake in the current study, a person with a body weight of 50 kg would have to drink about 2-3 L of 0.5% PE per day to get a similar effect. However, humans consume antioxidants (such as vitamins A, B, C and E as well as polyphenols, etc.) from various food sources everyday. Thus, a lower intake of 0.5% PE-mixed water may be effective in humans to ensure similar effects. Further, detailed investigation is certainly required to understand the role of catechins in humans.

In conclusion, we suggest that long-term administration of green tea catechins increases antioxidative defenses in the brain and facilitates adult-born hippocampal neurogenesis. These beneficial effects could be involved in improving spatial learning ability in rats, which is seen after administration of green tea.

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