学 位 論 文 の 要 旨

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学 位 論 文 名	Long-Term Administration of Green Tea Catechins Increases Antioxidative Actions and Enhances Neurogenesis in the Hippocampus of Rats
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論 文 内 容 の 要 旨

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, can cross the blood-brain barrier and prevents oxidative stress-induced neuronal apoptosis.

In the process of aging, oxidative stress is increased and induces a disorder of cellular function. The brain is vulnerable to oxidative damage due to its lower antioxidant capacity. Therefore, a balance between the cellular oxidative stress and the antioxidative defense in the brain is particularly important. We previously reported that long-term administration of green tea catechins reduces lipidperoxide (LPO) and reactive oxygen species (ROS) concentrations in brain and improves spatial cognition learning ability in rats. To understand the mechanisms, in this study we measured the expression and activities of brain antioxidative enzymes which are known to control the oxidant-antioxidant balance. In addition to antioxidative mechanism, hippocampal neurogenesis which plays an important role in learning of memory was also examined.

MATERIALS AND METHODS

This study protocol was designed in accordance with the "Guidelines for Animal Experimentation" of the Center for Integrated Research in Science, Shimane University.

Animals and diet: Five-weeks-old male Wister rats (n=32) were orally administered either green tea catechins (Polyphenon E, PE; 0.5% w/v) mixed with water or water alone for 26 weeks. The composition of PE is EGCG (63%), EC (11%), EGC (6%) and ECG (6%) was freshly prepared every other day, given to treated group animals in the drinking water.

Enzyme activity and gene expression analyses: Rats were anesthetized, brains were quickly removed and the cerebral cortex and hippocampus were separated for measuring antioxidative enzyme activity and their mRNA expression. A portion of brain tissues from hippocampus and cerebral cortex was homogenized with 0.32 mol/L sucrose buffer (pH 7.4) containing protease inhibitors. Then homogenates were centrifuged and the supernatant was used to analyze enzyme activities of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD). The other portion of the hippocampus and cerebral cortex was used to extract total RNA and cDNA synthesis. The mRNA expression of these antioxidative enzymes was measured by quantitative real-time PCR using SYBR Green PCR Master mix. 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.

Immunohistochemistry: To examine the role of PE on neurogenesis, 5-bromo-2'-deoxy uridine (BrdU, 50 mg/kg; i.p.) was injected for 5 consecutive days and stained for BrdU positive cells. The neuronal precursor proliferation and newborn cell survival were evaluated by counting BrdU-Neuronal Nuclei (NeuN) double positive cells in the dentate gyrus (DG) at the different time points (1 day and 5 weeks) after last BrdU injection. Brains were sliced coronally in 40 μm sections and every sixth section (240 μm apart) was used for staining. Rat anti-BrdU antibody and mouse anti-NeuN antibody were used as primary antibody and Alexa 633-conjugated goat anti rat IgG antibody and Alexa 488 conjugated goat anti mouse antibody were used as secondary antibody. Numbers of immunoreactive nuclei of BrdU-NeuN double positive cells were counted in the granule cell layer of DG.

RESULTS AND DISCUSSION

There was no significant difference in the volume of water or PE-mixed water intake between the groups. Based on the water volume intake, daily PE intake was approximately

131 ± 7.0 mg/rat in the 0.5% PE group. The final body weight did not differ between the groups.

The enzyme activities of CAT, GPx, GR, total SOD and MnSOD were significantly increased in the cerebral cortex (by 1.3, 1.4, 1.9, 2.1, 3.1 fold, respectively) and hippocampus (by 1.3, 1.2, 1.5, 2.1, 2.1 fold, respectively) of 0.5% PE-rats compared to the control group ($P<0.05$). It is suggesting that PE administration increases antioxidative action in brain. These changes might be involved in preventing age-related cognitive decline, since decrease lipid peroxide level improves cognitive ability. To confirm the antioxidative action in brain, we measured the mRNA level of antioxidative enzymes. The mRNA levels of CAT, GPx, GR and MnSOD increased in the hippocampus of 0.5% PE group (3, 1.2, 5 and 6.6 fold, respectively) than control group ($p<0.05$), suggesting that long-term administration of green tea catechins increases antioxidative defense especially in the hippocampus, which is an indispensable part for memory formation. The antioxidative action of PE can protect brain cell, which are highly vulnerable to oxidative damage during aging.

In addition to antioxidative action, a positive correlation between hippocampal neurogenesis and learning improvement has been reported in senescent rats. In this experiment, the immunohistochemical analyses revealed that the number of BrdU-NeuN double positive cells in the granule layer of DG were significantly increased in 0.5% PE group both at one day (control: 342.36 ± 60; 0.5 % PE: 568.51 ± 62, $P<0.05$) and at five weeks after the last BrdU injection (control: 267 ± 18; 0.5% PE: 393 ± 30, $P<0.05$). 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, $P=0.957$). It is suggesting that long-term PE administration increases neuronal precursor proliferation and newborn cell survival in DG of the rat hippocampus, which may be another mechanism in improving learning ability in PE-administered rats.

CONCLUSION

Taken together we suggest that long-term administration of green tea catechins increases antioxidative action and enhances neurogenesis in the hippocampus of rat. This is possibly related to the mechanisms involved in improving cognitive function of green tea catechins-administered rats.