学 位 論 文 の 要 旨

氏名　Abdul M. Haque

学 位 論 文 名　Long-term Administration of Green Tea Catechins Improves Spatial Cognition Learning Ability in Rats

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著 者 名　Abdul M. Haque, Michio Hashimoto, Masanori Katakura, Yoko Tanabe, Yukihiko Hara, Osamu Shido

論 文 内 容 の 要 旨

INTRODUCTION

The free-radical hypothesis suggests that increased production of lipid peroxide (LPO) and reactive oxygen species (ROS) exacerbates the neurodegenerative process. Oxidative stress induces the production of ROS and causes serious functional impairments including cognitive decline. A decrease in hippocampal LPO levels and/or an increase in antioxidative defense prevent and ameliorate oxidative stress-induced learning-impairment in rats.

Green tea is rich in polyphenols contained in the leaves and stems of the tea plant. The main polyphenolic components of green tea are (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and (-)-epicatechin gallate (ECG). EGCG, the major and most active component of green tea, acts as an antioxidant in a biological system. It can cross the blood brain barrier and prevents oxidative stress-induced neuronal apoptosis. Therefore, we investigated whether long-term administration of green tea catechins has any effects on spatial cognition learning ability as well as oxidative status in rats.

MATERIALS AND METHODS

This study was in compliance with the "Guidelines for Animal Experimentation" from the Center for Integrated Research in Science, Shimane University.

Animals, diet and experimental schedule: Five-weeks-old male Wistar rats were randomly divided into three groups and were orally administered either green tea catechins (Polyphenon E, PE) mixed with water or water alone for 26 weeks 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). PE solution, containing EGCG (63%), EC (11%), EGC (6%) and ECG (6%), was freshly prepared every other day. The rats were maintained with a 12:12 h dark-light cycle under controlled temperature (23 ± 2°C) and humidity (50 ± 10 % relative humidity), and given free access to a normal laboratory diet.

Radial maze learning ability: Five months after starting the PE administration, the rats' learning ability was tested by assessing their behavior in an 8-arm radial maze. Briefly, rats were trained to acquire a reward (food-pellet) at the end of each of 4 baited arms of the 8-arm radial maze. The performance involved two 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 weeks.

Tissue preparation: After completing the maze task, the rats were anesthetized and blood was collected. They were then sacrificed and the cerebral cortex and hippocampus were separated. Portions of the frontal cortex (100 mg) and hippocampus (50 mg) were 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/L leupeptin, 0.5 mg/L pepstatin, 0.5 mg/L aprotinin and 0.2 mmol/L phenylmethylsulfonyl fluoride using a Polytron homogenizer.

Measurements of antioxidative status: LPO concentrations were measured by the thiobarbituric acid reactive substance (TBARS) assay and calculated relative to a standard preparation of 1,1,3,3-tetraethoxypropane. Plasma total antioxidant activity was measured by the ferric

reducing antioxidation power (FRAP) assay. The levels of ROS were quantified from a dichlorofluorescein standard curve in methanol. Protein concentration was estimated using the method of Lowry et al., (1951).

RESULTS AND DISCUSSION

There were no significant differences in water volume intake and body weights among the 3 groups. Randomized two-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 ($F_{9,477} = 45.44$, $P < 0.0001$) and groups ($F_{2,106} = 22.24$, $P < 0.0001$) on the number of RMEs without a significant block × group interaction. Similarly, significant main effects of both blocks of trials ($F_{9,477} = 40.28$, $P < 0.0001$) and groups ($F_{2,106} = 9.35$, $P = 0.0002$) were observed on the number of WMEs, but with a significant block × group interaction ($F_{18,954} = 2.90$, $P < 0.0001$). Subtest analyses of the number of RMEs and WMEs demonstrated that the 0.1% PE and 0.5% PE-administered rats had lower RME scores than the controls, suggesting that long-term administration of PE improved spatial cognitive learning ability of rats.

When we investigated the oxidative biomarkers, lower concentrations of LPO and greater concentrations of FRAP of plasma were found in the PE-treated rats than in the controls. In hippocampus, the rats with preadministered PE had lower ROS levels than the controls. In addition, significant positive correlation between hippocampal TBARS concentrations and the number of RMEs and negative correlation between the plasma FRAP levels and the number of RMEs were found in block 10 of the radial maze task in the controls and the 0.5% PE-administered rats.

CONCLUSION

The present study suggests that chronic administration of green tea catechins improves spatial cognition learning ability by facilitating antioxidative defense in rats.