Effect of green tea catechin on prevention of age-related brain dysfunction

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Summary

We investigated the effect of long-term intake of green tea catechin on brain aging using mice with accelerated senescence (SAMP10). Age-related decline of learning ability was significantly prevented in mice that ingested green tea catechin (0.2 mg/ml). Epigallocatechin gallate (EGCG) was important for the prevention of brain dysfunction. Mice that ingested epigallocatechin (EGC) with gallic acid (GA) also had suppressed brain dysfunction, but not mice that ingested either EGC or GA. The binding ability of EGC to lipid membranes was increased in the presence of GA. The levels of oxidized DNA and lipid damage marker in serum were significantly lower in mice that ingested catechin than age-matched control mice. These observations suggest that anti-oxidative and membrane-binding activities of catechin are important for preventing senescence in the brain. DNA microarray data of the hippocampus in mice that ingested catechin is being analyzed.

Introduction

Various biological systems work in conjunction to maintain optimal brain function and cognitive ability. The brain is particularly susceptible to oxidative damage since it consumes roughly 20% of the oxygen used by the entire body, and because it contains high concentrations of phospholipids, which are especially prone to oxidative damage in the context of a high metabolic rate. Aging is the outcome of a balance between damage and repair, and is probably related to a multi-factorial process. Although a modest level of reactive oxygen species (ROS) generated under physiological conditions participate in cell signal transduction cascades to regulate cell growth and differentiation, in contrast, severe ROS cause oxidative damage to cellular DNA, protein and lipids. With aging, there is a significant and progressive increase in the level of oxidatively damaged DNA, protein and lipids in the brain and this free radical damage leads to the death of neurons. Endogenous antioxidants and antioxidative enzymes are engaged in the detoxification of ROS. In addition, numerous dietary antioxidants are thought to be involved in the antioxidative defense system.

We have found that the production of superoxide anion increased with aging in the brain of mice, rats and birds (Sasaki et al. 2008). The level was higher in the brain of senescence-accelerated mice (SAMP10) than the same-aged control mice (SAMR1) of normal longevity. Although the activity of superoxide dismutase (SOD) did not change, the activity of glutathione peroxidase was lower in the brain of SAMP10 at 12 months (Kishido et al. 2007). These results suggest that oxidative damage increased with aging. Actually, DNA oxidative damage in aged SAMP10 was higher than in the same-aged control mice (Unno et al. 2004, 2007). However, we previously found that green tea catechins (GT-catechin), potent antioxidants, decrease oxidative damage to DNA and suppress brain dysfunction in aged SAMP10 when ingested from between 1 and 12 months of age.

GT-catechin is composed of many kinds of catechins such as (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epicatechin (EC) (Fig. 1). To clarify the mechanism of GT-catechin in the brain, we examined whether the suppressive effect of GT-catechins on lowered learning ability was caused by an active component in GT-catechin or a summed ability of several catechins. Moreover, the level of oxidative damage in DNA and lipids was investigated in mice that had ingested catechins.

Materials and methods

Animals: All experimental protocols were performed in accordance with the guidelines for the care and use of laboratory animals of the University of Shizuoka. Male SAMP10/TaSlc (SAMP10) mice, which are senescence-prone, were purchased from Japan SLC Co., Ltd. (Shizuoka, Japan) and bred under conventional conditions in a temperature- and humidity-controlled room with a 12-h light/dark cycle. Experimental mice had free access to a normal diet (CE-2; Clea Co Ltd, Tokyo, Japan) and tap water containing green tea catechins. Catechin water was freshly prepared twice a week. Control mice were given a normal diet and tap water ad libitum.

Green tea catechins and experimental design: Polyphenon 70S (Polyphenon 70S, Mitsui Norin Co. Ltd., Tokyo, Japan) contains about 70% GT-catechin and no caffeine. GT-catechin consists of 31.7% EGCG, 15.7% EGC, 10.0% ECG and 8.5% EC. The remaining portion consists of 4.5% gallocatechin gallate, 1.0% catechin gallate, and some other catechins from green tea. Sunphenon EGCg (Taiyo Kagaku Co. Ltd., Yokkaichi, Japan) consists of 95.1% EGCG and 2.8% ECG. Sunphenon EGC (Taiyo Kagaku Co. Ltd.) consists of 81.4% EGC and 7% EC. EGCG and EGC are abundant catechins in green tea. Since the concentration of EGCG and EGC in GT-catechin solution (0.2 mg/ml) was 0.06 and 0.03 mg/ml, respectively, both the effects of EGCG and EGC were compared with that of GT-catechin at these concentrations. A group of mice consumed GT-catechin in water at a concentration of 0.20 mg/ml from 2 to 12 months. The other groups of mice consumed EGCG (0.06 mg/ml), EGC (0.03 mg/ml), or gallic acid (GA, 0.02 mg/ml), respectively. Another group of mice consumed both EGC and GA. Control mice consumed water. The levels of hexanoyllysin (HEL) and 8-oxodeoxyguanosine (8-oxodG) in serum were measured as markers of oxidative damage in lipid and DNA, respectively.

Memory acquisition test: A step-through passive avoidance task was carried out using 11-month-old mice as described previously (Unno et al. 2004, 2007). In brief, when a mouse entered the dark chamber from the light chamber, the door was closed and an electric foot-shock was delivered at 50 μA for 1 s (SGS-003, Muromachi Kikai Co., Ltd., Tokyo, Japan). Acquisition of the avoidance response was judged as successful if the mouse remained in the light chamber for 300 s. The trial was repeated until the mouse satisfied the acquisition criterion within five trials. The time that a mouse could not stay in the light chamber, i.e., the time spent in the dark chamber in a 300-s trial, was recorded. This result from successive trials was summed for each mouse to give a measure of the time required for learning not to enter the light chamber (i.e., "learning time").

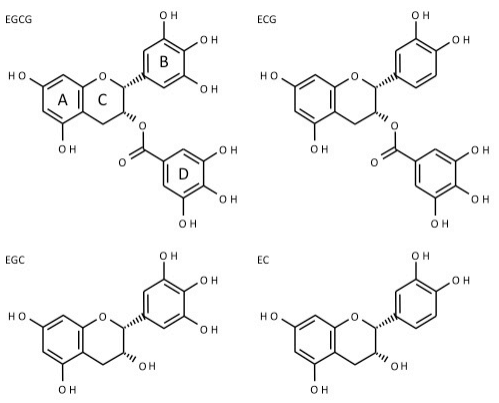

Fig. 1 Structure of GT-catechin

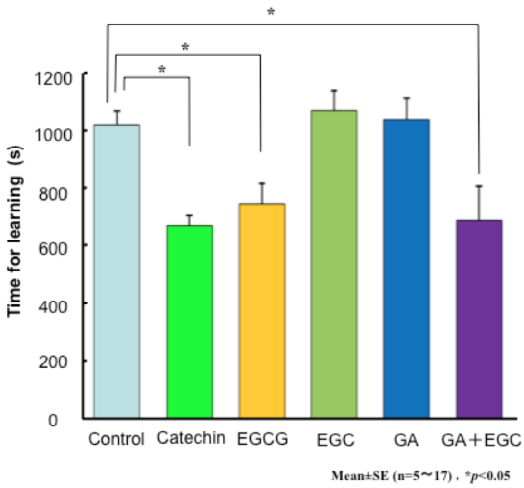

Fig. 2 Effect of catechins on learning ability

Results and discussion

Learning ability: The time for learning not to enter the dark room was measured at 11 months of age using a step-through passive avoidance task. A shorter learning time implies higher learning ability. The learning times of mice that ingested GT-catechin and EGCG were significantly shorter than age-matched control mice (Fig. 2). The learning time of mice that ingested EGCG was as similar to that of mice that ingested GT-catechin. When mice ingested either EGC or GA, mice showed a learning time as long as that of control mice. However, the learning time was significantly shorter in mice that ingested EGC with GA. These results suggest that the active component in green tea catechins is EGCG. In addition, the ingestion of EGC with GA also effectively suppresses cognitive dysfunction. Recent studies on EGCG metabolism indicate that it is first hydrolyzed to EGC and GA in the intestine (Takagaki and Nanjo, 2010; Schantz et al. 2010). These results suggest that EGCG and its metabolites, EGC and GA, are similarly effective in cognitive function.

Membrane binding ability and antioxidative activity: As the concentrations of EGCG and EGC used in this experiment were 0.13 and 0.10 mM, respectively, the difference in concentration was only a small or negligible reason for the difference in functional activity. To unravel the mechanism by which brain dysfunction is inhibited by catechins, the relation between catechin structure and brain function was considered. The affinity of EGCG to lipid bilayers is higher than that by EGC (Nakayama et al. 2000; Sun et al. 2009). In this study, the effect of GA on binding ability of EGC to phospholipid bilayers was measured. The binding ability of EGC was higher in the presence of GA than in its absence while the ability of EGCG was higher than that of EGC with GA. The increased binding ability of EGC to biomembranes in the presence of GA might affect, in part, the incorporation of EGC into the brain.

Next, the effect of catechin administration on oxidative stress was also considered. Green tea catechins are generally regarded as antioxidants because they all have multiple hydroxyl substituents on the A, B, C and/or D ring (Fig. 1), and the reducing activities of gallocatechin (EGCG and EGC) are similarly strong (Lambert and Elias, 2010). The levels of HEL and 8-oxodG were significantly lower in mice that ingested catechins than age-matched control mice. The levels tended to be lower in mice that ingested GT-catechin, EGCG and EGC + GA than in mice that ingested either EGC or GA. The anti-oxidative activity of catechins *in vivo* may be important in preventing cognitive function.

Active component in green tea catechin: When we measured the learning time of SAMR1 as a positive control mouse, the times were 524 ± 53 s at 11 months of age and 679 ± 80 s at 14 months of age, respectively (Unno et al. 2004). That result suggests that the learning ability of SAMP10 mice that ingested GT-catechin or EGCG was similar to that of SAMR1. The learning ability using a step-through passive avoidance task indicated that the ability of mice that ingested EGCG was similar to that of mice that ingested GT-catechin. While learning ability did not improve in mice that ingested EGC, it improved in the presence of GT. The metabolites EGCG, EGC and GA may thus be effective in cognitive function. The observed functional activity of green tea catechins (GT-catechin = EGCG, EGC + GA >> EGC) indicates that EGCG was an active component of GT-catechins in preventing brain dysfunction.

How long should green tea catechins be ingested? When should the ingestion of green tea catechins start? The results indicate that an ingestion period >5 months (1-11, 3-11, 6-11, 3-9, and 1-6) was significantly effective while ingestion periods of 2 and 3 months (9-11 and 3-6) were slightly effective (Unno et al. 2011). We predicted that mice that ingested EGCG only when young (i.e., 1-6 months) might not fully suppress cognitive dysfunction if the accumulation of damage starts from adulthood or middle age. However, the ingestion of EGCG for 1-6 months was as effective as ingestion for 3-9 and 6-11 months, suggesting that oxidative damage accumulated from a young age. In SAMP10 mice, 8–9 months is considered to be the middle of the life span. On the other hand, body weight increased until about 4 months; thereafter, body weight was maintained at a plateau level suggesting that an age above 4 months was a mature adult. Therefore, an intake period above 5 months accounts for about 1/3 of the lifespan of these mice, suggesting that the intake of catechin ought to start at adulthood or middle age at the latest.

As the generation of superoxide anion was higher in SAMP10 than in age-matched SAMR1 (Sasaki et al. 2008), the level of carbonyl protein, a marker of oxidative damage, was higher in SAMP10 than in age-matched SAMR1. Oxidative damage in some important proteins seems to be critical for nerve cells. For example, glutathione peroxidase, a major antioxidative enzyme, exhibited low activity in aged SAMP10, but the decreased activity was significantly improved by the ingestion of GT-catechin (Kishido et al. 2007). The decreased activity of antioxidative enzymes with aging seems to be a reason for the increased oxidative damage. However, further investigation is needed to resolve the mechanism of GT-catechin in the brain.

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