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Green Tea Catechin Causes an Weight Loss in Transgenic Mice Over-expressing Carboxyl Terminus of Amyloid Precusor Protein

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Abstract – Amyloid β (Aβ) has been reported to have an effect on the induction of oxidative stress that involves the functional and structural abnormalities in Alzheimer's disease. As a role of a radical scavenger, a green tea treatment was found to have some inhibitory effect on the neurodegenerative process. The aim of this study was to determine if green tea catechin (GTC) reduces in transgenic model. To test this, transgenic mice carrying neuron-specific enolase (NSE) controlled C-terminus (105) of APP (APP-C105) were created and treated them with a low and a high dose of GTC for 6 months. Herein, we concluded that transgenic mice expressing NSE/APP-C105 were successfully created and the GTC-treated group exhibited significant reduction in body weight. Thus, GTC might be a good prevention of obesity or good treatment for AD patient.

Keywords □ Alzheimer transgenic, Green tea catechin, Weight loss

INTRODUCTION

The carboxyl-terminus of APP is cleavages by either α-secretase or β-secretase, and it includes Aβ as well as the transmembrane and intracellular domains. These are subsequently cleavage at two sites by γ-secretase to produces either the Aβ residue 1-40 (Aβ 40) or a longer, more amyloidogenic form that contains the residues 1-42 (Aβ42) (Hass et al., 1992; Citron et al., 1996; Klafuki et al., 1996; Mulphy et al., 1999). The g-secretase complex consists of at least four proteins such as presenilins (PSs), nicastrin, APH-1, and PEN2, and thus producing Aβ42. The neurofibrillary tangle (NFT) is a neuronal inclusion of the microtuble-binding protein tau and is composed of phosphorylated and ubiqutinated tau aggregations forming a β-pleated sheet structure. Tau dysfunction and filamentous tau aggregates are the key markers in the pathology of Alzheimer's disease (AD) that display NFT. Both Aβ-42 and NFT are known to be the main neuropathological characteristics of AD.

The carboxyl terminal fragments of the βAPP include the 42-amino acid and the 58-62 adjacent amino acids (APP/C100-C104), which is a mutant protein responsible for causing the AD neuropathology. In a previous study, transgenic mice carrying the neuron-specific enolase (NSE) promoter that controlled the C-terminal of the βAPP (NSE/APP-C105) were created (Lim, et al 2005). This transgenic line was shown to be responsible for the memory impairment and elevated production of Aβ-42 in the brain at age of 10 months. In parallel, APP-C105 overexpression resulted in the modulation of the Aβ-42 level, γ-secretase activity, GSK3β-binding proteins including PS1, tau, and β-catenin in the brains of the transgenic mice relative to the non-transgenic mice.

Polyphenols including flavonoids are classified into anthocyanins, anthozanthine, and anthoxantines. The anthoxantines are further divided into several categories including flavones, flavans, flavonols, flavanols, and isoflavones (Weinreb, et al 2004). They have remarkable antioxidant activity due to the large number of phenolic hydroxyl groups on the aromatic ring, which is condensed to a heterocyclic ring and attached to a second aromatic ring that confers its antioxidant activity (Van De Craen, et al 1999).

There is a lot of study in the effect of green tea catechin (GTC) on weight reduction on blood parameters, but it has not been characterized Alzheimer's disease-model animals. In our previous study, treadmill exercise resulted in a reduction of Aβ-

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42 deposits, an improvement in behavior function, thereby restoring normal concentration of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglyceride. The aim of this study was to created transgenic mice expressing NSE-controlled APP-C105, to administer GTC to transgenic mice and determine if there were any efficient reduction of the AD-related body weight. To accomplish this, 6 month old transgenic mice were given a low and high doses of GTC in their drinking water for 6 months, and a subset of the groups was then tested the body weight.

MATERIALS AND METHODS

Gene Construction

A pNSE/APP-C105 plasmid was constructed. The pNSE-Splice was constructed by inserting the NSE sequence into the pTet-Splice (Gibco, BRL), where the tetracycline operator sequence (Tet) had been eliminated by digestion with the *XhoI*/*SpeI* enzymes. The rat NSE promoter was amplified by PCR, using the sense primer (5'-CGTCG ACTATGGTGG TATGG CTGA-3') with a nucleotide 37-55, and the antisense primer (5'-TCGAG GACTG CAGAC TCAG-3') with a nucleotide 1786-1804 using pNSE/CAT (Forss et al., 1990) as the template. The primers contained an added recognition sequence for *SalI* and *SpeI*, to the 5' and 3' end of the PCR products, respectively. The pNSE/CAT was a kind gift from Dr. J Gregor-Sutcliffe at the Research Institute of Scriffs Clinic. The pNSE-T used in this study was inserted into the amplified NSE product (1777-bp).

Transgenic mice

The transgenic mice expressing the NSE-controlled APP-C105 was established by back crossing the founder mice with a parental strain of C57BL/6 mice in this group (Lim, et al 2005). Transgenic and non-transgenic littermates were handled in an accredited Korea FDA animal facility in accordance with the AAALAC International Animal Care policy (Accredited Unit-Korea Food and Drug Administration: Unit Number-000996), which was based on NIH guidelines. The mice was housed in cages under a strict light cycle (lights on at 06:00h and off at 18:00h). In addition, all the mice were given a standard irradiated chow diet (Purina Mills, Inc) *ad libitum*, and maintained in a specified pathogen-free state.

Water maze test

The mice were used for the water maze tests as previously described (Manthey et al., 2001; Hwang et al., 2004). The tests were performed using the SMART-CS (Panlab, Barcelona Spain) program, placed in an experimental room, with a window, air-conditioning and tables. This experiment was carried out in a 1.5 m diameter plastic circular pool of water at 22°C and the visual field was obstructed by the addition of powdered milk. Mice were pretrained by allowing them to swim to a round shape platform (diameter 12 cm) submerged 1 cm beneath the surface. The escape latencies, escape distances, swimming speeds and swimming patterns of the mice were monitored by a computer, using the SMART-LD program, which was connected to a camera mounted to the ceiling directly above the pool. A 60 sec habituation trial was performed in order to verify their ability to swim. The mice that were given 5 training trials during which their latency to locate the hidden platform was measured up to a maximum of 60 sec. The mice were placed on the platform by the experimenter when they failed to find the platform within the maximum time. The training schedule consisted of two trials per day, over 5 test days, and each trial was assessed by the ability of the mouse to reach the platform within 60 sec. A second trial was performed at least 5 min after the first. The platform location was kept constant during the training period. After each trial, the mice remained on the platform for 30 sec. On day 6, the mice were subjected to three probe trials, where they would swim for 60 sec, with no platform in the pool. In this test, each of the two training and three prove trials were initially started from the right side of the water pool, and on the opposite site of the pool in the second time. The patterns of searching, the number of the times (escape latency), the distances swan (escape distance) and the swimming (velocity) speeds to the precise former location were all recorded. All the trials were stored on videotape for subsequent analysis.

Western blot analyses

The brains from the transgenic and non-transgenic littermates were solubilized with 1% Nonidet P-40 in 150 mM NaCl, 10 mM Tris HCl buffer (pH 7.5), and 1 mM EDTA supplemented with a protein inhibitor mixture (Roche), which was followed by centrifugation at 10,000xg and 4°C for 10 min. For western blotting, samples were run on 4~20% gradient polyacrylamide gels, and transferred to nitrocellulose membranes. The membranes were then incubated with the antibodies [Aβ-42 (anti-amyloid β protein monoclonal antibody, Chemicon Int.). The proteins were then transferred onto nitrocellulose membrane (Amersham Biosciences, UK) for 2hr and 45V in transfer buffer (25 mM Trizma-base, 192 mM glycine and 20%

methanol). The efficiency of transfer and equal protein loading was determined by staining the membrane with Amido Black Staining Solution (Sigma, MI) and gel with Coomassie Blue. Appropriate dilutions of the primary antibodies were added to the membranes and allowed to hybride overnight at 4°C. The antibody was removed and membrane washed three times in a solution containing 10 mM Trizma-base (pH7.6), 150mM NaCl, and 0.05% Tween-20 for 10min. This was followed by incubation with horseradish peroxidase-conjugated anti-secondary antibody for 1hr at room temperature. The membrane was washed again as described above and developed using an enhanced chemiluminescence detection system (Amersham Bioscience, UK). The results were quantitated using Image Analyzer System (Estman Kodak 2000_{MM}, USA) and expressed as fold increase over control values.

Materials

Polyphenon 60 (product number, P1204; Lot 043K1137) extracted from green tea was purchased from Sigma-Aldrich (Missouri, USA). Polyphenon 60 contains 70% of catechin consisted of 34% (-)-Epigallocatechin gallate (EGCG), 16.7% (-)-Epigallocatechin (EGC), 8.7% (-)-Epicatechin gallate (ECG), 7.3% (-)-Epicatechin (EC), 2.8% (-)-gallocatechin gallate, and 0.5% (-)-catechin gallate (Fig. 1). Structures of catechin in polyphenon 60 are shown in Fig. 1 (Ikeda, et al 2003).

Treatments

Seven-month old males and females were separated into four groups [11-15 (sex ratio, about 1:1) per group]; three groups of transgenic mice and a non-transgenic group. Of the groups, two groups were treated with a low (0.02%, Tg-1) and high doses (0.2%, Tg-2) of GTC in their drinking water, and another two groups were used as the controls of normal water in transgenic (Tg-0) and non-transgenic littermates (Non-Tg). A subset of groups was then maintained for 7 months with or without GTC in their drinking water. The doses and periods were chosen based on a report, showing that 0.02% GTC from 1 to 6 and 12 months was effective in improving the learning and memory in aged 12-month-old senescence-accelerated mice (SAMP10) (Unno, et al 2004). Water containing GTC and normal water were prepared freshly every other day. Seven months later, 4 groups were sacrificed for analysis. Here, 0.02% dose relates to the amount of 400mg GTC that might to present in a human dietary supplement with a total amount of 2 liter water during a single day. It is because mouse was taken 1mg GTC in 5ml of water per day.

Statistical analysis

The tests for significance were performed using a One-Way ANOVA analysis of the variance (SPSS for Windows, Release 10.01 Standard Version, Chicago, IL). All the values are reported as the mean ± standard deviation (s). A P value <0.05 was considered significant.

RESULTS

Strategy for construction of the pNSE/hAPP-C105

A. pNSE/APP-C105 plasmid. APP-C105 was placed under the control of the NSE gene promoter. APP-C105 was cloned (Fig. 1A) and this was further cloned into T-vector (Fig. 1B). Finally, NSE-controlled APP-C105 was obtained (Fig. 1C).

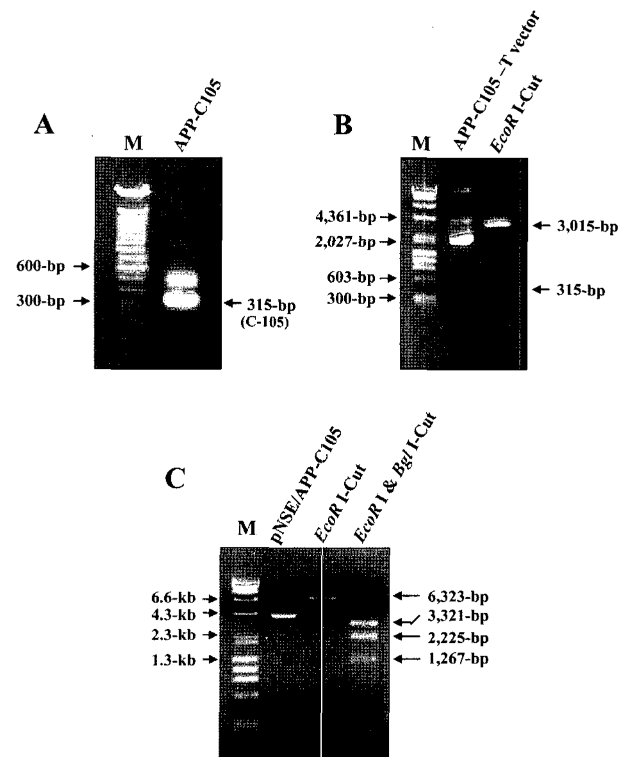

Fig. 1. Construction of pNSE/APP-C105. A. The APP-C105 sequence was amplified by PCR, with a full-length of APP695sw (Y00264) as the template. The primers used for the amplification as follows; sense primer, 5'- GACTA GTATG TCTGA AGTGA AGATG GAGTC-3' (corresponding to the nucleotide 1921-1942 of APPsw), and antisense primer, 5'-GTCTA GTTCT GCATC TGCTC-3' (corresponding to the nucleotide 2218 - 2238 of APPsw). B. The amplified APP-C105 product was cloned into the pGEM-T (pAPP-C105-T). C. the APP-C105 fragment obtained by the digestion of pAPP-C105-T with SpeI, was cloned into the SpeI site of the pNSE-splice (pNSE/APP-C105). The CMVAPP695sw was a kind gift from Dr. Tae-Wan Kim at Columbia University.

Identification of constructed pNSE/hAPP-C105 gene sequence

pNSE/APP-C105 plasmid (Fig. 2Aa) combining the NSE promoter was constructed. The APP-C105 sequence was amplified by the PCR, with a full-length of APP695sw as a template. Primers used for the amplification were; sense primer, 5'-TCTAG ATCGC GATGC TG-3' (corresponding to nucleotide 143-154 of APPsw), antisense primer, 5'-GTCTA GAGTC TAGTT CTGCA TC-3' (corresponding to nucleotide 2223-2238 of APPsw). The constructed pNSE/hAPP-C105 gene was verified for the gene presence and orientation using PCR and amino acid sequencing of NSE promoter and hAPPC105 gene (Fig. 2Ab). After microinjection of this construct, APP-C105 transgene was identified by DNA-PCR and Southern blotting. The founder mice (4 week of age) were examined for the presence of the transgenes by DNA-PCR (Fig. 2Ba) and Southern blot analysis (Fig. 2Bb). Two founder mice (#4840 and #4844) were then backcrossed to the parental C57BL/6 strain in order to establish the independent lines. Both of the founder mice transmitted the transgene into genomes of their offspring of both genders in approximately a 50% hemizygous Mendelian inheritance manner.

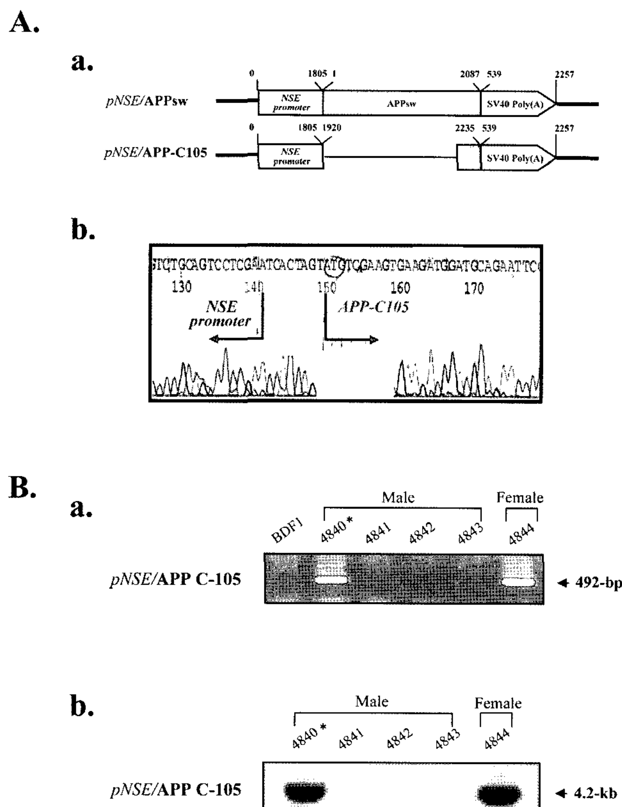

Fig. 2. Conformation of DNA sequence. A. Construction. B. Verifying the sequence between NSE promoter and APP-C105.

Memory impairment and enhanced level of Aβ42

The In order to assess the behavioral deficits in APP-C105 transgenic mice, swimming patterns were measured using water maze tests. On the sixth-day, all the mice underwent three proof trials, where they swam in the pool for 60 sec with the platform removed. The swimming patterns across to the former platform location in the transgenic mice were significantly different than that observed in their non-transgenic littermates (Fig. 3A). Therefore, the overexpression of the APP-C105 gene in the brains of the transgenic mice leads to behavior deficits. After the behavioral test, the 10-month old transgenic and non-transgenic littermates were sacrificed, and the expression levels of Aβ-42 and β-actin were measured (Fig. 3B). The western

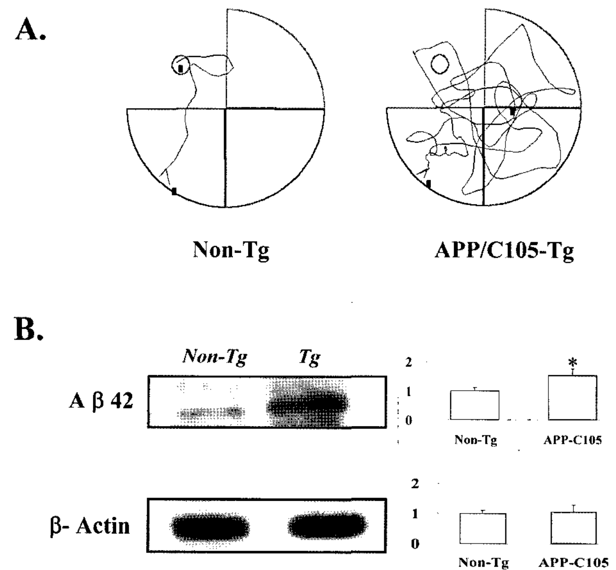

Fig. 3. Memory impairment and Enhanced level of Aβ42. A. Swimming patterns. These patterns show the mice crossing to the former platform location in the water maze. The NSE/APP-C105-transgenic lines show a consistent trend toward longer escape latencies and distances than their non-transgenic littermates from 9 months of age whereas the swimming velocities of the transgenic mice and their non-transgenic littermates were similar. Nine to 11, mice with ratio of about 1 (male) : 1 (female) per group in duplicate were studied, using the water maze tests. The values are represented as a mean ± SD. *p<0.05 versus the non-transgenic littermates. A. Aβ-42 expressions. B. Western blot. The lysates from the brains of the transgenic mice were mixed with the primary antibody to the anti-β-amyloid 42. Western blot was performed from the brains of transgenic mice and non-transgenic littermates. Anti-β-amyloid 42 antibody detected Aβ-42 deposition (Fig. 3B).

blot revealed a significantly higher level of the total Aβ-42 induction in the transgenic mice than their non-transgenic littermates [F(22.73)=0.001, $p<0.05$]. The β-actin levels were similar between the transgenic and non-transgenic littermates.

GTC leads to a body weight levels

Using the transgenic mice the level of body weight was measured in order to determine of GTC (Fig. 4) could reduces body weight. The body weight level was significantly lower in Tg-1 and Tg-2 groups than Tg-0 group (Fig. 5).

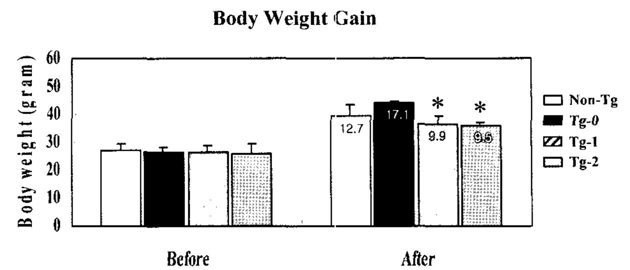

Fig. 5. Measurement of body weight. Before: 6-minths mice. After: 12-months-age of mice after treatment during 6-months

DISCUSSION

In this study, transgenic mice, over-expressing NSE-controlled APP-C105 were successfully created and used to address the hypothesis that GTC might improve the body weight in this study. Induced level of Aβ-42 in transgenic brain was significantly lowed in body weight. This approach offers a critical insight, which might have an impact on developing a strategy for preventing AD.

Upon examining the behavior using a water maze test, the 9 and 10-months-old transgenic mice, had longer escape latencies when crossing to the former platform location than the non-transgenic mice. This suggests that this behavior represents a lack of memory in finding the former platform location. In the mice with behavior defects, the elevated level of Aβ-42 was observed, but there was no plaque formation in the cortex and hippocampus of the transgenic mice. Indeed, the occurrence of behavior defects before plaque formation was common in all the transgenic mice overexpressing the PS1 mutations (Paech et al., 1997) or the mutant APP, including those not developing amyloid plaque (Paech et al., 1997; Fitzpatrick et al., 2002). It should be noted that the soluble Aβ-42 concentration correlated directly with the levels of memory impairment. It was recently reported that the soluble Aβ-42 concentration in human brains correlated better with the severity of the disease than the plaque itself (McKenna et al., 2001; Means et al., 1972; McDonnel et al., 2001). Moreover, the soluble Aβ-42 concentration appears to correlate with the cognitive impairment (Means et al., 1972; McDonnel et al., 2001; Abe et al., 2000; Liu et al., 2001). Therefore, the mice express-

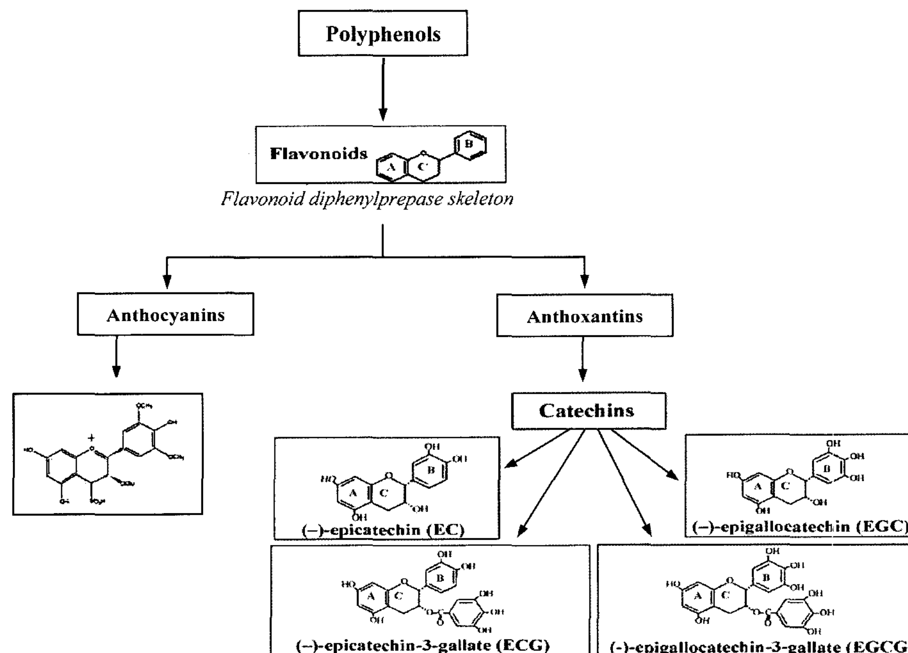

Fig. 4. Structure of Green tea catechin.

mutant- and APP-transgenic mice.

Why then should have a reduction in body weight by treatment of GTC? There are three possible explanations. i) Aβ-42-induced oxidative stress is reduced through the scavenging of hydroxyl radicals of GTC, and its reduction contributes the changes in the weight loss. ii) GTC can lower the cholesterol level by increasing the number of LDL-C receptors (LRP). GTC mediates LRP to maintain the balance between the degradation and production of Aβ (Goto, et al 2002). Moreover, LRP was up regulated by GTC through the sterol-regulated element binding protein (SREBP-1) (Bursill, et al 2001). Therefore, this receptor might act to clear cholesterol from the blood. iii) The GTC treatment resulted in a decrease in cholesterol absorption. Indeed, EGCG reduced the plasma TC, and non-HDL-C by inhibiting the level of cholesterol absorption associated with mild fat mal-absorption, which alters the micelle structure (Raederstorff, et al 2003).

In conclusion, this paper provides the first experimental evidence that a GTC treatment given to AD-model transgenic mice leads to the weight loss, which might contributes to benefits for human health.

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