

## A Comparison between Extract Products of *Magnolia officinalis* on Memory Impairment and Amyloidogenesis in a Transgenic Mouse Model of Alzheimer's Disease

Young-Jung Lee<sup>1,2,3</sup>, Dong-Young Choi<sup>1,2,3</sup>, Sang Bae Han<sup>1,2,3</sup>, Young Hee Kim<sup>4</sup>, Ki Ho Kim<sup>4</sup>, Yeon Hee Seong<sup>5</sup>, Ki-Wan Oh<sup>1,2,3</sup> and Jin Tae Hong<sup>1,2,3,\*</sup>

<sup>1</sup>College of Pharmacy, <sup>2</sup>Medical Research Center, and <sup>3</sup>CBITRC, Chungbuk National University, Cheongju 361-763, <sup>4</sup>R&D Center, Bioland Ltd., Cheonan 330-863, <sup>5</sup>College of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Republic of Korea

### Abstract

The components of *Magnolia officinalis* have well known to act anti-inflammatory, anti-oxidative and neuroprotective activities. These efficacies have been sold many products as nutritional supplement extracted from bark of *Magnolia officinalis*. Thus, to assess and compare neuroprotective effect in the nutritional supplement (Magnolia Extract™, Health Freedom Nutrition LLC, USA) and our ethanol extract of *Magnolia officinalis* (BioLand LTD, Korea), we investigated memorial improving and anti-Alzheimer's disease effects of extract products of *Magnolia officinalis* in a transgenic AD mice model. Oral pretreatment of two extract products of *Magnolia officinalis* (10 mg/kg/day in 0.05% ethanol) into drinking water for 3 months ameliorated memorial dysfunction and prevented A $\beta$  accumulation in the brain of Tg2576 mice. In addition, extract products of *Magnolia officinalis* also decreased expression of  $\beta$ -site APP cleaving enzyme 1 (BACE1), amyloid precursor protein (APP) and its product, C99. Although both two extract products of *Magnolia officinalis* could show preventive effect of memorial dysfunction and A $\beta$  accumulation, our ethanol extract of *Magnolia officinalis* (BioLand LTD, Korea) could be more effective than Magnolia Extract™ (Health Freedom Nutrition LLC, USA). Therefore, our results showed that extract products of *Magnolia officinalis* were effective for prevention and treatment of AD through memorial improving and anti-amyloidogenic effects via down-regulating  $\beta$ -secretase activity, and neuroprotective efficacy of *Magnolia* extracts could be differed by cultivating area and manufacturing methods.

**Key Words:** *Magnolia*, Tg2576, Alzheimer's disease, Natural product

### INTRODUCTION

The bark of the root and stem of *Magnolia* family has been used in oriental traditional medicine to various diseases such as neurosis, anxiety, stroke, fever, gastrointestinal disorders, allergic disease and headache (Watanabe *et al.*, 1983; Song *et al.*, 1989; Kuribara *et al.*, 1998). To define these effects, researchers isolated many bioactive components such as honokiol, obovatol, magnolol and 4-O-methylhonokiol which have anti-inflammatory (Lin *et al.*, 2007; Munroe *et al.*, 2007; Zhou *et al.*, 2008), neuroprotective (Lin *et al.*, 2006) and anti-oxidative effect (Fujita and Taira, 1994; Chen *et al.*, 2007; Dikalov *et al.*, 2008). We previously reported that 4-O-methylhonokiol have memory improving functions in several different animal models; scopolamine-infused model (Lee *et al.*, 2009), A $\beta$ -infused animal model (Lee *et al.*, 2010; Lee *et al.*, 2011c), systemic

LPS-induced animal model (Lee *et al.*, 2012b), and presenilin 2 mutant (Lee *et al.*, 2011a), Swedish APP, and Tg2576 AD mouse models (Lee *et al.*, 2012d). In addition, we reported that obovatol also have memory improving effects in systemic LPS-induced animal model (Choi *et al.*, 2012a) and Tg2576 AD mouse models (Choi *et al.*, 2012b). Hou *et al.* (2000) reported that co-treatment of magnolol and honokiol may enhance *in vivo* hippocampal acetylcholine release. The co-treatment of magnolol and honokiol was reported to prevent age-related learning and memory impairment in senescence-accelerated-prone 8 (SAMP8) mice (Matsui *et al.*, 2009). In addition, neuroprotective effect in magnolol or honokiol via anti-oxidative effect was also reported by many researchers (Lin *et al.*, 2006; Cui *et al.*, 2007; Hoi *et al.*, 2010). These studies indicate that extract of *Magnolia officinalis* (ME) could have various neuroprotective effects as well as many health benefit effects. For

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\*Corresponding Author

E-mail: jinthong@chungbuk.ac.kr

Tel: +82-43-261-2813, Fax: +82-43-261-8273

this reason, several extract products of *Magnolia* family have been sold as dietary supplement for benefit of health, and used by many people in the worldwide.

Alzheimer's disease (AD) is the most common cause of dementia, accounting for 50 to 75% of all cases (Blennow *et al.*, 2006; Ferri *et al.*, 2009). AD is pathologically characterized by intraneuronal neurofibrillary tangles and extraneuronal senile plaques, which are primarily composed of aggregates of the microtubule-associated protein tau, and the amyloid beta ( $A\beta$ ) peptide, respectively (Castellani *et al.*, 2007).  $A\beta$  is proteolytically produced from the amyloid precursor protein (APP) by proteases, called  $\beta$ - or  $\gamma$ -secretases (De Strooper and Annaert, 2000). Because the deposition of  $A\beta$  contributes to progressive degeneration and loss of neurons in the brain, the procedure of  $A\beta$  production and deposition has been critically targeted as therapeutic strategies against AD (Golde *et al.*, 2006).  $\beta$ -Secretase known as  $\beta$ -site APP cleaving enzyme 1 (BACE1) is considered as a major target for preventing amyloidogenesis in the therapy and/or prevention of AD (Yan *et al.*, 1999; Lin *et al.*, 2000). BACE1 is upregulated by a variety of risk factors such as hypoxia, mitochondrial dysfunction and oxidative stress (Cole and Vassar, 2007). Thus, inhibitory effect of these risk factors is also considered as targets for prevention and treatment of AD. Transgenic models of AD have been made by expressing human APP, presenilin 1 or presenilin 2 gene, and they showed age-related cognitive impairment, and concurrent deposition of  $A\beta$  plaque (Hsiao, 1998; Janus and Westaway, 2001; Duff and Suleman, 2004). The Tg2576 mice overexpress a mutant human  $A\beta$  precursor protein and develop impairment of spatial memory and deposition of  $A\beta$  plaque in cortex and hippocampus (Hsiao *et al.*, 1996; Chapman *et al.*, 1999; Westerman *et al.*, 2002). Thus, Tg2576 mice have been used to investigate memorial improving and anti-amyloidogenic effect of various new developed drugs.

In this study, we investigated and compared the memory improving and anti-amyloidogenic effects of two ME products on the Tg2576 mice. We found that both two ME products prevented memory impairment through prevention of amyloidogenesis in the Tg2576 mice.

## MATERIALS AND METHODS

### Magnolia extract

Our ethanol extract of *Magnolia officinalis* (BL-ME) was supplied by Bioland Ltd. (Chungnam, Korea) as an ethanol extract of 99.6% purity. The bark of *Magnolia officinalis* was purchased from Kyung-Dong Market, Seoul, Korea, and taxo-

nomically identified by Dr. Bang Yeon Hwang at the Research Institute of Drug Resource, Chungbuk National University (Cheongju, Korea). A voucher specimen (CBNU 9301) was deposited at the Herbarium of Chungbuk National University, Chungbuk, Korea. The bark of *Magnolia officinalis* was dried in the shade at room temperature and stored in a dark, cold room until use. The air-dried bark of *Magnolia officinalis* (3 kg) was cut into pieces and extracted twice with 95% (v/v) ethanol (four times as much as the weight of the dried plants) for 3 days at room temperature. After filtration through the 400 mesh filter cloth, the filtrate was re-filtered through filter paper (Whatman, No. 5) and concentrated under reduced pressure. The BL-ME contained 1.24% magnolol, 1.81% honokiol, 1.24% 4-O-methylhonokiol and others (unpublished data). *Magnolia* Extract™ (HFN-ME) was purchased from Health Freedom Nutrition, LLC (Reno, Nevada, USA), and contained 4.5% of honokiol and magnolol.

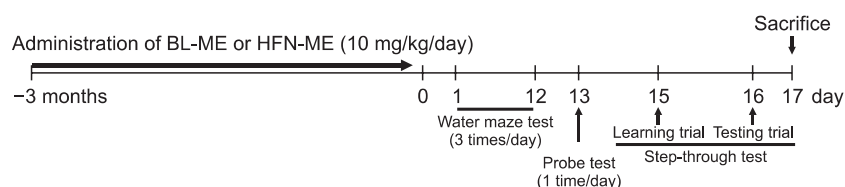
In our previous studies, treatment of 1 mg/kg/day 4-O-methylhonokiol showed memory improving and neuroprotective effect in various animal models (Lee *et al.*, 2010; Choi *et al.*, 2011; Lee *et al.*, 2011a; Lee *et al.*, 2011c). Therefore, we used 10 mg/kg/day ME products including these components to administrate toward *in vivo* animal models in this study. The ME products dissolved in 0.05% ethanol were added to drinking water and mice were allowed access to it for 3 months *ad libitum* before induction of memory impairment.

### Alzheimer's disease mouse model

Transgenic lines, overexpressing APP the K670M/N671L mutant gene, were established by backcrossing the founder mice with the parental strain of C57BL/6-SJL mice as described elsewhere (Hsiao *et al.*, 1996). 12-months-old transgenic mice and age-matched normal C57BL/6 mice were purchased from Taconic Farms (Germantown, NY, USA), and were maintained in accordance with the Institutional Animal Care and Use Committee (IACUC) of Laboratory Animal Research Center at Chungbuk National University, Korea (CBNU-404-12-01). All mice were housed in a room that was automatically maintained at 21–25°C and relative humidity (45–65%) with a controlled light-dark cycle. Experimental scheme was shown in Fig. 1.

### Water maze test

The water maze test is also a widely accepted method for memory test, and we performed this test as described by Morris (1984). Maze testing was performed by the SMART-CS (Panlab, Barcelona, Spain) program and equipment. A circular plastic pool (height: 35 cm, diameter: 100 cm) was filled with



**Fig. 1.** Experimental scheme for administering two ME products to Tg2576 mice. Tg2576 mice were treated with BL-ME or HFN-ME (10 mg/kg/day) into drinking water for 3 months. Then, water maze test was performed at 3 times per day for 12 days. A probe test was performed 24 hr after the water maze test. Then, passive avoidance step-through test was 48 hr after the probe test. After step-through test, sacrifice was performed and molecular biological assays were performed. BL-ME: Ethanol extract of *Magnolia officinalis* from Bioland LTD, HFN-ME: Extract product of *Magnolia officinalis* from Health Freedom Nutrition LLC.

milky water kept at 22-25°C. An escape platform (height: 14.5 cm, diameter: 4.5 cm) was submerged 0.5-1 cm below the surface of the water in position. On training trials, the mice were placed in a pool of water and allowed to remain on the platform for 10 sec and were then returned to the home cage during the second-trial interval. The mice that did not find the platform within 60 sec were placed on the platform for 10 sec at the end of trial. They were allowed to swim until they sought the escape platform. These trials were performed in single platform and in three starting positions of rotational starting. Escape latency, escape distance, swimming speed and swimming pattern of each mouse was monitored by a camera above the center of the pool connected to a SMART-LD program (Panlab, Barcelona, Spain).

### Probe test

A probe trial in order to assess memory consolidation was performed 24 hr after the water maze tests. In this trial, the platform was removed from the tank, and the mice were allowed to swim freely. For these tests, percentage time in the target quadrant and target site crossings within 60 sec was recorded. The time spent in the target quadrant is taken to indicate the degree of memory consolidation that has taken place after learning. Swimming pattern of each mouse was monitored by a camera above the center of the pool connected to a SMART-LD program described above.

### Passive avoidance performance test

The passive avoidance test is also widely accepted as a simple and rapid test method for measuring memory capacity. The passive avoidance response was determined using a "step-through" apparatus (Med Associates, Inc., Georgia, VT, USA) that is consisted of an illuminated and a dark compartments (each 20.3×15.9×21.3 cm) adjoining each other through a small gate with a grid floor, 3.175 mm stainless steel rod set 8 mm apart. One day after water maze test, training trial was performed. The mice were placed in the illuminated compartment facing away from the dark compartment. When the mice moved completely into the dark compartment, it received an electric shock (1 mA, 3 sec duration). Then the mice were returned to their home case. At one day later, the mice were placed in the illuminated compartment and the latency period to enter the dark compartment defined as "retention" was measured. The time when the mice entered in the dark compartment were recorded and described as step-through latency. The retention trials were set at a limit of 180 sec of cut-off time.

### Brain collection and preservation

After behavioral test (step through test), animals were perfused with saline under ether inhalation. The brains were immediately collected and separated into cortical and hippocampal regions. All the brain regions were immediately stored at -80°C, and used to biological assay.

### Immunohistochemistry

Mice were anesthetized with ether. While general anesthesia, the mice received intracardiac perfusion with 20 ml of saline, followed by 50 ml of phosphate-buffered saline (PBS) containing 4% paraformaldehyde. After perfusion, the brain were removed from the skull and post-fixed for 2-4 hr in the same fixative, and were then cryoprotected overnight in 30%

sucrose prepared in PBS. Serial coronal sections of brain (40  $\mu$ m) were cut with a freezing microtome. Immunohistochemical staining was performed using the avidin-biotin peroxidase method. The sections were incubated overnight at 4°C with anti-A $\beta_{1-42}$  (1:2000 dilution, Covance, Berlely, CA, USA). After washing in PBS, the sections were incubated in biotinylated goat anti-mouse IgG (1:2000 dilution, Vector Laboratories, Burlingame, CA, USA) for 1 hr at room temperature. The sections were subsequently washed and incubated with avidin-conjugated peroxidase complex (ABC kit, 1:200 dilution, Vector Laboratories) for 30 min followed by PBS washing. The peroxidase reaction was performed in PBS using 3, 3'-diaminobenzidine tetrahydrochloride (DAB, 0.02%) as the chromogen. Finally, the sections were rinsed, mounted on poly-glycine-coated slides, dehydrated, and cover-slipped for light microscopy and photography.

### Western blotting analysis

Tissues were homogenized with lysis buffer [50 mM Tris pH 8.0, 150 mM sodium chloride (NaCl), 0.02% sodium azide, 0.2% sodium dodecyl sulfate (SDS), 1 mM phenylmethanesulphonyl fluoride, 10  $\mu$ g/ml aprotinin, 1% IGAPEL CA-630, 10 mM sodium fluoride, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM ethylene glycol tetraacetic acid (EGTA) and 0.5% sodium deoxycholate] and centrifuged at 15,000×g for 15 min. Equal amount of proteins (40  $\mu$ g) were separated on a SDS/10 and 15% polyacrylamide gel and then transferred to a polyvinylidene difluoride membrane (GE Water and Process Technologies). Blots were blocked for 1 hr at room temperature with 5% (w/v) non-fat dried milk in Tris-buffered saline [10 mM Tris (pH 8.0) and 150 mM NaCl] solution containing 0.05% Tween 20. The membrane was then incubated for 3 hr at room temperature with specific antibodies: anti-APP (1:500 dilution, ABR-affinity Bioreagents), anti-BACE1 (1:500 dilution, Sigma) and anti- $\beta$ -actin (1:2000 dilution, Santa Cruz Biotechnology) were used. The blots were then incubated with the corresponding conjugated anti-rabbit and anti-mouse immunoglobulin G-horseradish peroxidase (Santa Cruz Biotechnology). Immunoreactive proteins were detected with the ECL Western blotting detection system.

### Measurement of A $\beta_{1-42}$

Lysates of brain tissue were obtained through protein extraction buffer containing protease inhibitor. A $\beta_{1-42}$  levels were determined using specific ELISA Kit (Immuno-Biological Laboratories Co., Ltd., Takasaki-Shi, Gunma, Japan). In brief, 100  $\mu$ l of sample was added into the pre-coated plate and was incubated for overnight at 4°C. After washing each well of the pre-coated plate with washing buffer, 100  $\mu$ l of labeled antibody solution was added and the mixture was incubated for 1 hr at 4°C in the dark. After washing, chromogen was added and the mixture was incubated for 30 min at room temperature in the dark. Finally, the resulting color was assayed at 450 nm using a microplate absorbance reader (Sunrise™, TECAN, Switzerland) after adding stop solution.

### Statistical analysis

Statistical analysis of the data was carried out using analysis of variance (ANOVA) for repeated measures followed by Dunette's post-hoc analysis using GraphPad Prism 4 software (Version 4.03, GraphPad software, Inc.).

## RESULTS

### ME products ameliorated learning and memory impairment in Tg2576 mice of AD model

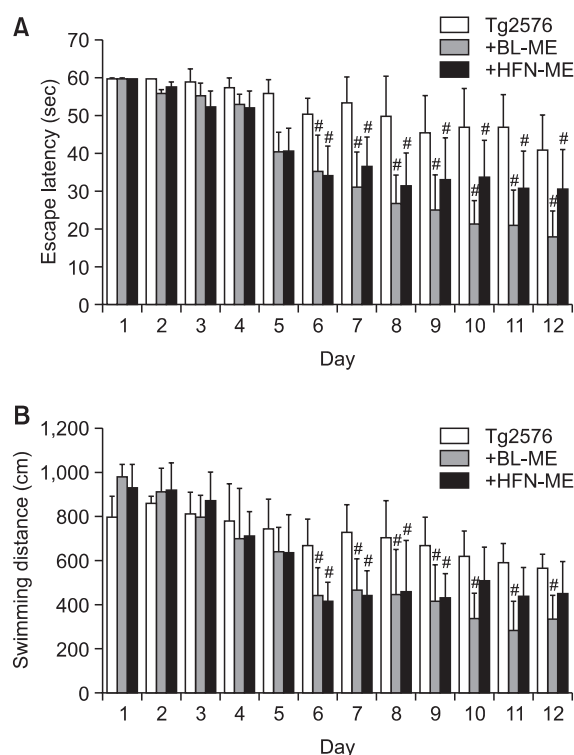
To investigate and compare preventive effect of ME products against memory impairment and  $A\beta_{1-42}$  depositions in the AD model, 12 months old Tg2576 transgenic mice were continuously administrated BL-ME or HFN-ME at a dose of 10 mg/kg/day daily for 3 months, and then compared memory deficiency with the non-treated Tg2576 mice using the water maze test, probe test and step-through test as shown in Fig. 1. The Tg2576 mice were trained for three trials per day during 12 days when most of Tg2576 mice could get maximum memory for escaping platform. Then, the Tg2576 mice were analyzed to locate and escape onto the platform, and their spatial learning scores were recorded. Significant differences by memory improving effect on Tg2576 mice were shown after 5 days, and sufficient learning of platform for probe test was required 12 days. The mice exhibited gradual decreased in escape latency by the training. However, the escape latency of Tg2576 mice was not much reduced than that of ME-treated Tg2576 mice. Oral treatment of all ME products (10 mg/kg/day daily for 3 months) significantly ameliorated memory dysfunction in the AD model mice. Statistical analysis of data from day 6 to day 12 showed the significance of memory improving effect by ME products. Escape latency of the BL-ME treated

group ( $20.7 \pm 9.5$  sec,  $p=0.028$ ) and the HFN-ME treated group ( $30.6 \pm 9.8$  sec,  $p=0.046$ ) on day 11 was shorter than that of the non-treated Tg2576 group ( $46.7 \pm 8.6.0$  sec) (Fig. 2A). Swimming distance of the BL-ME treated group ( $280.0 \pm 131.9$  cm,  $p=0.031$ ) and the HFN-ME treated group ( $438.5 \pm 130.47$  cm,  $p=0.063$ ) on day 11 was shorter compared with that of the non-treated Tg2576 group ( $588.12 \pm 88.18$  cm) (Fig. 2B). However, there was no significant difference on average speed between the non-treated group and the two ME-treated groups (data not shown). Although all of ME products showed to ameliorate memorial dysfunction in memorial ability test using water maze test, BL-ME treatment was more effective than the HFN-ME treatment.

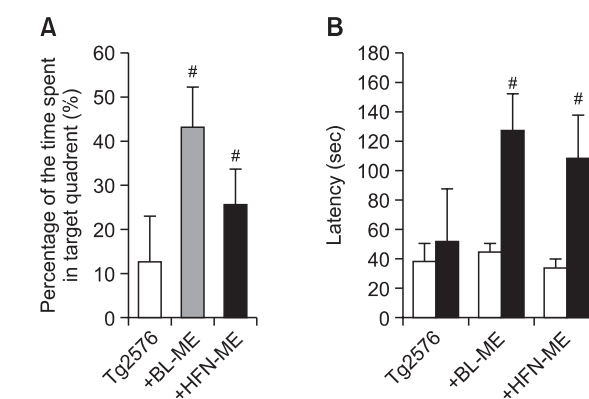
After the water maze test, we performed a probe test to analyze maintenance of memory. During the probe test, the time spent in the target quadrant on the BL-ME treated group ( $43.1 \pm 8.9\%$ ,  $p=0.002$ ) and the HFN-ME treated group ( $25.6 \pm 8.2\%$ ,  $p=0.043$ ) was significantly increased compared to the non-treated Tg2576 group ( $12.5 \pm 10.4\%$ ) (Fig. 3A). Although all of ME products showed to ameliorate memorial dysfunction in memorial ability test using probe test, BL-ME treatment was also more effective than the HFN-ME treatment. We then evaluated learning and memory capacities by the passive avoidance test by step-through method. In the passive avoidance test, there was no significant difference on the learning trial. However, in test trial, step-through latency of the non-treated Tg2576 mice group ( $51.1 \pm 36.6$  sec) significantly increased to  $114.3 \pm 27.8$  sec ( $p=0.005$ ) by BL-ME treatment and  $94.9 \pm 31.2$  sec ( $p=0.02$ ) by HFN-ME treatment (Fig. 3B), and effects of two ME products did not show significant difference in step-through test.

### ME products prevented $A\beta_{1-42}$ accumulation in the mice brain of AD model

Many studies reported that  $A\beta_{1-42}$  accumulation, which



**Fig. 2.** Memory improving effect of two ME products on water maze test in Tg2576 mice. Training trial was performed 3 times per day for 12 days. Escape latency (A) and swimming distance (B) to arrive at platform was automatically recorded in water maze test. Values are presented as mean  $\pm$  S.E.M. from 10 mice. #Significant difference from non-treated Tg2576 mice ( $p<0.05$ ). BL-ME: Ethanol extract of *Magnolia officinalis* from Bioland LTD, HFN-ME: Extract product of *Magnolia officinalis* from Health Freedom Nutrition LLC.



**Fig. 3.** Memory improving effect of two ME products on probe test (A) and step-through test (B) in Tg2576 mice. The time spent in target zone in a probe test (A) conducted following the completion of training was recorded. Values are presented as mean  $\pm$  S.E.M. from 10 mice. To perform passive avoidance test (B), the mice were given electric shock when entered dark room for training on learning day. After one day of the learning day, the retention time in illuminated compartment was recorded. Values are presented as mean  $\pm$  S.E.M. from 10 mice. #Significant difference from non-treated Tg2576 mice ( $p<0.05$ ). BL-ME: Ethanol extract of *Magnolia officinalis* from Bioland LTD, HFN-ME: Extract product of *Magnolia officinalis* from Health Freedom Nutrition LLC.

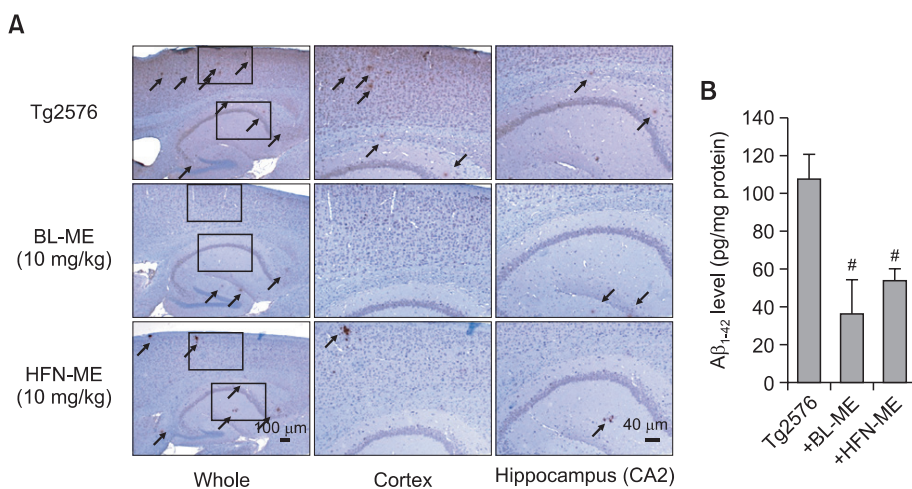


is thought to be a major cause of AD, occurred in the brain of Tg2576 mice. Thus, we investigated and compared anti-amyloidogenic effects of two ME products. The immunohistochemical analysis using  $A\beta_{1-42}$  specific antibody showed  $A\beta_{1-42}$  deposition in the cortical and hippocampal regions of Tg2576 mice brain. However, administration of two ME products significantly decreased accumulated  $A\beta_{1-42}$  plaques in the brain (Fig. 4A). In particular, accumulated  $A\beta_{1-42}$  on brain of BL-ME-treated Tg2576 mice was slightly lower than that of HFN-ME-treated Tg2576 mice. Then, to confirm the decrease of  $A\beta_{1-42}$  plaque by immunohistochemical analysis, we performed quantitative analysis of  $A\beta_{1-42}$  level using ELISA kit. In parallel with the immunohistochemical result, high  $A\beta_{1-42}$  level on brains of Tg2576 mice was detected as previous reported (Lee *et al.*, 2012d), and administration of two ME products

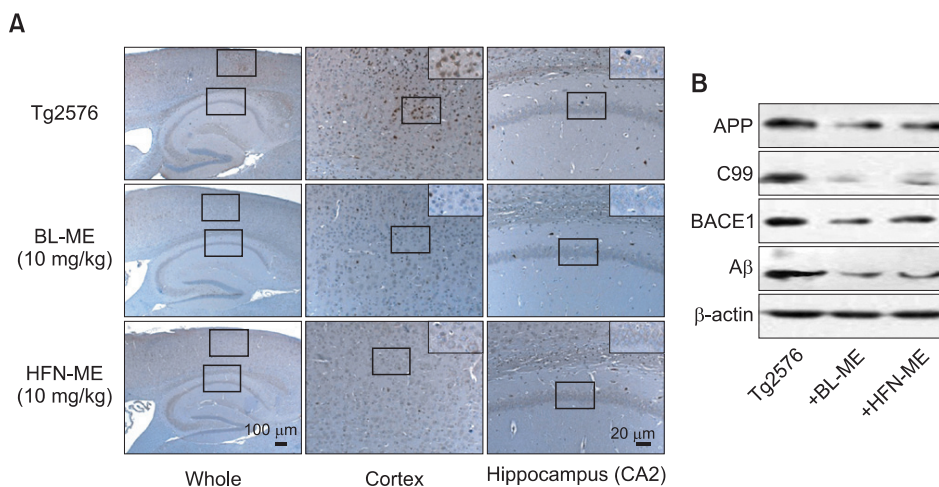
significantly decreased these levels (Fig. 4B). In particular, administration of BL-ME slightly more decreased  $A\beta_{1-42}$  levels than that of HFN-ME (Fig. 4B).

**ME products inhibited amyloidogenesis in the mice brain of AD model**

$A\beta_{1-42}$  is produced from sequential proteolytic cleavage of APP by  $\beta$ - and  $\gamma$ -secretase.  $\beta$ -Secretase is the first step in the production of the  $A\beta_{1-42}$  (Selkoe, 1998). In parallel with the reduced  $A\beta_{1-42}$  level in Tg2576 mice treated with ME products, the number of BACE1 specific antibody-reactive cells were also significantly reduced in cortex and hippocampus of Tg2576 mice treated with ME products (Fig. 5A). To confirm reduction of accumulated  $A\beta$  level and BACE1 reactive cells by two ME products, we investigated the expression of



**Fig. 4.** Inhibitory effect of two ME products on Ab accumulation in the Tg2576 mice brains.  $A\beta$  accumulation in the brain was determined using immunohistochemical analysis by  $A\beta_{1-42}$  specific antibody. Elevated accumulation of extracellular  $A\beta_{1-42}$  was observed by immunohistochemical method as described in the materials and methods (A). Arrow indicated the accumulation of  $A\beta_{1-42}$ . The effect of two ME products on  $A\beta_{1-42}$  level in the brain was measured by ELISA kit (B). Values are presented as mean  $\pm$  S.E.M. from 5 mice. <sup>#</sup>Significant difference from non-treated Tg2576 mice ( $p < 0.05$ ). BL-ME: Ethanol extract of *Magnolia officinalis* from Bioland LTD, HFN-ME: Extract product of *Magnolia officinalis* from Health Freedom Nutrition LLC.



**Fig. 5.** Anti-amyloidogenic effect of two ME products in the brains of Tg2576 mice. Expression of  $\beta$ -secretase were investigated in the cortex and hippocampus regions (A), and expression of APP, C99, BACE1 and  $A\beta$  were also detected in the brain (B). BL-ME: Ethanol extract of *Magnolia officinalis* from Bioland LTD, HFN-ME: Extract product of *Magnolia officinalis* from Health Freedom Nutrition LLC.

full-length APP, BACE1, its processing product C99 and A $\beta$ . Both expression level of full-length APP, C99, BACE1 and A $\beta$  increased in brain of Tg2576 mice, and these levels were reduced by treatment with ME products (Fig. 5B). Although both ME products showed anti-amyloidogenic effects, BL-ME treatment was more effective than the HFN-ME treatment.

## DISCUSSION

The bark of the *Magnolia officinalis* has been used in traditional herbal medicines in Korea, China and Japan (Iwasaki *et al.*, 2000; Luo *et al.*, 2000). The bark of *Magnolia officinalis* is known as a rich source of biologically active compounds. It has been reported to have at least 255 different ingredients, such as alkaloids, coumarins, flavonoids, lignans, neolignans, phenylpropanoids and terpenoids (Ito *et al.*, 1982; Tachikawa *et al.*, 2000). The *Magnolia* bark in the traditional medicines has been known to have anti-cancer effects (Choi *et al.*, 2002), anti-inflammatory effects (Kang *et al.*, 2008) and anti-oxidant actions (Kong *et al.*, 2000). In particular, the *Magnolia* bark showed anti-stress, anti-anxiety (Weeks, 2009), anti-depressant (Xu *et al.*, 2008), anti-Alzheimer and anti-stroke effects (Lee *et al.*, 2010; Lee *et al.*, 2011b). Recently, we found that oral administration of ethanol extract of *Magnolia officinalis* (2.5, 5 or 10 mg/kg) ameliorated the A $\beta_{1-42}$  (0.5  $\mu$ g/mouse, i.c.v.)-induced memory impairments and inhibited apoptotic neuronal cell death as well as BACE1 expression (Lee *et al.*, 2010), and also inhibited memory impairments and A $\beta_{1-42}$  accumulation on the human APP695-expressing Tg2576 mice (Lee *et al.*, 2012c).

In this study, to confirm the preventive effect of ME products and compare neuroprotective effect of two ME products on memory impairment of human APP695-expressing Tg2576 mice, we respectively administrated previous maximum concentration (10 mg/kg/day) of two ME products for 3 months, and found the memory improving effect of all ME products in Tg2576 mice of AD model through behavior tests such as water maze test, probe test and step-through test. Although all ME products were effective for preventing memory impairment, escape latency of the BL-ME treated group on day 11 of water maze test was twice shorter than that of HFN-ME, and the time spent on probe test was also twice longer than that of HFN-ME (Fig. 2A and 3A). Thus, memory improving effect of BL-ME was slightly higher than that of HFN-ME. Administration of two ME products inhibited A $\beta_{1-42}$  production and accumulation in Tg2576 mice. In particular, A $\beta_{1-42}$  level in BL-ME treated group was about 60% lower than that of untreated group and about 20% lower than that of HFN-ME (Fig. 4B), indicating that anti-amyloidogenic effect of BL-ME was slightly effective compare to that of HFN-ME. These differences were believed to be contributed by ratio of components or producing district of *Magnolia* trees as previously reported (Lee *et al.*, 2011b). These results suggest that all ME products could prevent occurrence and progress of AD.

The ME and its components such as magnolol, honokiol, obovatol and 4-O-methylhonokiol have been also reported to have neuroprotective effect through several mechanisms. Co-treatment of magnolol and honokiol was reported to enhance hippocampal acetylcholine release (Hou *et al.*, 2000). It was reported that co-treatment of magnolol and honokiol in SAMP8 mice prevented age-related learning and memory

impairment (Matsui *et al.*, 2009). The neuroprotective effect of magnolol or honokiol was reported to occur via its anti-oxidative effect (Lin *et al.*, 2006; Cui *et al.*, 2007; Hoi *et al.*, 2010). Lee *et al.* (2012a) reported that obovatol has neuroprotective and anti-oxidative effect in SH-SY5Y neuroblastoma cells. Recently, we also found that obovatol has neuroprotective effect in human APP-overexpressed Tg2576 model and LPS-induced AD animal model (Choi *et al.*, 2012a). We reported that 4-O-methylhonokiol has memory improving and neuroprotective functions in several different animal models such as scopolamine-infused model (Lee *et al.*, 2009), LPS-induced AD model (Lee *et al.*, 2012b), A $\beta$ -infused model (Lee *et al.*, 2010; Lee *et al.*, 2011c), presenilin 2 mutant (Lee *et al.*, 2011b), Swedish APP (APPsw) AD mouse models (Choi *et al.*, 2011), and human APP overexpressed (Tg2576) AD mouse models (Lee *et al.*, 2012d). We found several functional mechanisms such as inhibition of A $\beta_{1-42}$ -induced ROS generation and neuronal cell death (Lee *et al.*, 2010), inactivation of  $\beta$ -secretase and ERK pathway (Lee *et al.*, 2011a) as well as inhibition of cytokine release and NF- $\kappa$ B activity by 4-O-methylhonokiol in different animal models (Lee *et al.*, 2012b). Thus, many studies reported anti-amyloidogenic effect of many components of ME through its multiple mechanisms, suggesting that crude extract of *Magnolia officinalis* including these components can also show memory improving and neuroprotective functions in AD patient as well as elderly human. In particular, it is possible that synergistic effect in these components induce more effective neuroprotective functions as well as memory improving effects. For this reason, several extract products of *Magnolia* family have been sold as dietary supplement by several companies.

Although pharmacodynamics and pharmacokinetics of whole components of *Magnolia* extract were not investigated yet, pharmacodynamics and pharmacokinetics of single component was reported by several investigators. Lin *et al.* (2011) reported that the magnolol was predominant in the liver, kidney, brain, lung and heart after intravenous (i.v.) or oral administration to Sprague-Dawley rats. Ten minutes after magnolol (5 mg/kg, i.v.) administration to rat, brain concentrations of magnolol showed no significant differences among various regions (cerebral cortex, olfactory bulb, hippocampus, striatum, cerebellum, brain stem and rest of brain) (Tsai *et al.*, 1996). We found that orally administered 4-O-methylhonokiol in ICR mice rapidly disappears from the blood and is distributed into brain rapidly (less than 1 hr after treatment). In addition, we found that the 4-O-methylhonokiol could be accumulated into brain, and about 50-100 ng/ml may be effective dose in the brain (unpublished data). Pharmacokinetic profile in Tg2576 mice supposes to be similar with ICR mice. Thus, we can assume that the similar results could be obtained in Tg2576 mice. Further, we will investigate bioavailability and pharmacokinetic studies in the Tg2576 mice. In addition, several ME products are recommended to be taken as 1.5-5 mg/kg/day from each company such as Health Freedom Nutrition LLC (Reno, Nevada, USA), Allergy Research Group LLC (Alameda, California, USA), and HealthAid Ltd (London, UK). Thus, we believe that dosage of 10 mg/kg/day is appropriate for analyzing memory improving and anti-amyloidogenic effects of two ME products.

In summary, two ME products ameliorated memory impairment in Tg2576 mice, and effectively reduced A $\beta$  accumulation via BACE1 expression. In addition, memory improving

and anti-amyloidogenic effect of BL-ME was slightly more effective compared to that of HFN-ME. These data suggest that ME is a strong potential candidate for treatment of AD.

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