Regular Article

The Additive Effects of Low Dose Intake of Ferulic Acid, Phosphatidylserine and Curcumin, Not Alone, Improve Cognitive Function in APPswe/PS1dE9 Transgenic Mice

Michiaki Okuda,^{*,a,b} Yuki Fujita,^{a,b} and Hachiro Sugimoto^c^aDepartment of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University; 46–29 Shimoadachi-cho, Sakyo-ku, Kyoto 606–8501, Japan; ^bGreen Tech Co., Ltd.; 1–7–7 Yaesu, Chuo-ku, Tokyo 103–0028, Japan; and^cFaculty of Life and Medical Sciences, Doshisha University; 1–3 Tatara Miyakodani, Kyotanabe, Kyoto 610–0394, Japan.

Received April 18, 2019; accepted July 11, 2019

Alzheimer's disease (AD) is the most common form of dementia and its prevention and treatment is a worldwide issue. Many natural components considered to be effective against AD have been identified. However, almost all clinical trials of these components for AD reported inconclusive results. We thought that multiple factors such as amyloid β ($A\beta$) and tau progressed the pathology of AD and that a therapeutic effect would be obtained by using multiple active ingredients with different effects. Thus, in this study, we treated ferulic acid (FA), phosphatidylserine (PS) and curcumin (Cur) in combination or alone to APPswe/PS1dE9 transgenic mice and evaluated cognitive function by Y-maze test. Consequently, only the three-ingredient group exhibited a significant improvement in cognitive function compared to the control group. In addition, we determined the amounts of $A\beta$, brain-derived neurotrophic factor (BDNF), interleukin (IL)-1 β , acetylcholine and phosphorylated tau in the mouse brains after the treatment. In the two-ingredient (FA and PS) group, a significant decrease in IL-1 β and an increasing trend in acetylcholine were observed. In the Cur group, significant decreases in $A\beta$ and phosphorylated tau and an increasing trend in BDNF were observed. In the three-ingredient group, all of them were observed. These results indicate that the intake of multiple active ingredients with different mechanisms of action for the prevention and treatment of AD.

Key words Alzheimer's disease; ferulic acid; phosphatidylserine; curcumin

INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disorder, is the most common form of dementia, characterized by senile plaques composed of amyloid- β ($A\beta$) peptide¹ and neurofibrillary tangles of tau protein.² The prevention and treatment of AD are ongoing concerns worldwide, and many natural components considered effective against AD have been identified.^{3,4} For example, ferulic acid (FA; Fig. 1A) is a phenolic molecule found in various fruits and vegetables and has antioxidant properties.⁵ In addition, FA inhibits $A\beta$ production *via* down-regulation of amyloid precursor protein (APP), β -secretase and γ -secretase⁶; inhibits β -secretase activity⁷ and $A\beta$ aggregation⁸; enhances expression of protein phosphatase 2A, which contributes to tau dephosphorylation,⁹ promotes clearance of phosphorylated tau *via* autophagy¹⁰; exhibits neuroprotective action^{11,12}; activates acetylcholine receptor¹³; and induces neural proliferation by increasing the levels of cyclic adenosine monophosphate response element binding protein (CREB) phosphorylation and brain-derived neurotrophic factor (BDNF) levels.¹⁴ Phosphatidylserine (PS; Fig. 1B) is a major phospholipid that constitutes approx. 20% of the total phospholipid mass of human cellular membranes.¹⁵ PS is required for healthy neural cell membranes and their proper functioning.^{16–19} As the brain ages, the PS content of neural cell membranes decreases, leading to a decline in the function of receptors, a decrease in neurotransmitter synthesis, and synaptic loss. Studies have reported that PS administration improves these conditions, in addition to cognitive function.^{20,21} PS also

promotes acetylcholine release,^{22–24} decreases the acetylcholinesterase (AChE) level,²⁵ maintains *N*-methyl-D-aspartate (NMDA) receptor,²⁶ and contributes to $A\beta$ clearance through exosome secretion.²⁷ Curcumin (Cur; Fig. 1C) is a natural polyphenol. It is a yellow-colored spice derived from turmeric (*Curcuma longa*) and has antioxidant and anti-inflammatory properties. It reportedly inhibits $A\beta$ production *via* APP maturation²⁸; α -secretase activation²⁹; β -secretase down-regulation³⁰; β -secretase inhibition^{31,32}; down-regulation of presenilin 1, which is a main subunit of γ -secretase³³; inhibition of γ -secretase activity³⁴; and modulation of $A\beta$ precursor protein trafficking.³⁵ Cur also inhibits $A\beta$ aggregation^{8,36} and promotes $A\beta$ clearance *via* up-regulation of degrading enzymes³⁷ and induction of autophagy.³⁸ In addition, Cur also reduces hyperphosphorylated tau *via* down-regulation³⁹ and inhibition³² of glycogen synthase kinase 3 β (GSK3 β) and inhibition of cyclin-dependent kinase 5,⁴⁰ inhibits tau aggregation,^{41,42} exhibits some kinds of neuroprotective effects,^{43–45} and promotes neurogenesis *via* histone acetylase inhibition⁴⁶ and BDNF expression.⁴⁷ As a result, the incidence of AD in the United States is higher compared to India, where Cur is consumed on a daily basis.⁴⁸ Cur has been demonstrated to improve memory function in nondementia adults.⁴⁹ However, almost all clinical trials on its effects on AD have reported negative or inconclusive results so far.⁵⁰ Therefore, it seems that using a single active ingredient cannot provide therapeutic effects against AD, because multiple factors such as $A\beta$ and tau are involved in the progress of AD pathology; instead, therapeutic effects might be obtained by using multiple active

* To whom correspondence should be addressed. e-mail: okuda.michiaki.33r@st.kyoto-u.ac.jp

Fig. 1. Structures of FA (A), PS (B), and Cur (C)

FA, ferulic acid; PS, phosphatidylserine; Cur, curcumin.

ingredients with different effects.

Since no significant improvement in cognitive function is observed by the use of FA, PS, or Cur alone, in this study, we investigated the adding and synergistic therapeutic effects of a mixture FA, PS, and Cur on cognitive dysfunction in APPswe/PS1dE9 transgenic mice.

MATERIALS AND METHODS

Test Compounds FA derived from rice bran was purchased from TSUNO Co., Ltd. (Katsuragi, Japan). PS derived from soy was purchased from NOF Corporation (Tokyo, Japan). Cur was purchased from OMNICA Co., Ltd. (Tokyo, Japan).

Treatment to APPswe/PS1dE9 Transgenic Mice APPswe/PS1dE9 transgenic mice were obtained from the Jackson Laboratory (Bar Harbor, ME, U.S.A.), and maintained by crossing the mice with B6C3F1 mice. Genotyping was performed using the PCR method recommended by the Jackson Laboratory, and hemizygote mice were selected for the treatment. Mice were maintained in a regulated environment (temperature $24 \pm 3^\circ\text{C}$; humidity $50 \pm 10\%$; 12-h inverted light-dark cycle) and allowed free access to food and tap water. In the two-ingredient group, FA and PS were mixed into the MF basal diet (Oriental Yeast Co., Ltd., Tokyo, Japan) at 0.05% each, and orally administered to mice ($n=6$). In the three ingredients group, FA, PS and Cur were mixed to the diet at 0.05, 0.05 and 0.01%, respectively, and orally administered to the mice ($n=7$). In the control group, the MF diet alone was orally administrated ($n=7$). The administration period was three months (from 6 to 9 months of age). Separately, the MF diet alone or the diet which was mixed with 0.2% Cur was orally administered to the mice ($n=12$, each group), and the MF diet alone or the diet which was mixed with 0.2% FA or 0.2% PS was orally administered to the mice ($n=5$, each group) in the same way as above. The experimental procedures involving mice and their care were conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee (authorization number; 16-56-1, 16-56-3) and those of the Japanese Pharmacological Society.

Y-Maze Test The working memory of the mice was evaluated using the Y-maze test at the start of the treatment and at 4th, 8th and 12th-weeks of the treatment. Each mouse was placed at one arm of a Y-maze, which has three arms 30 cm in length with equal angles between all arms. The mice were allowed to move freely in the maze for 8 min, and the sequence and number of arm entries were recorded. Spontaneous alternation behavior, which is used as a measure of spatial memory, was defined as entry into all three arms on consecu-

tive choices. The percentage of spontaneous alternations was calculated as follows:

$$\frac{\text{Number of spontaneous alternations}}{\text{Number of total arm entries} - 1} \times 100$$

Protein Extraction from Brain Tissue At the end of treatment, all mice were sacrificed under deep anesthesia with 100 mg/kg sodium pentobarbital (Kyoritsu Seiyaku Corp., Tokyo, Japan) administered intraperitoneally, and the whole brains were removed for biochemical analysis. The brain tissues were homogenized in five or 10 volumes (w/v) of $2\times$ RIPA buffer (Nacalai Tesque Inc., Kyoto, Japan) with 0.5 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich Co., St. Louis, MO, U.S.A.), $1\times$ PhosSTOP (Roche, Basel, Switzerland), and 1% protease inhibitor cocktail (Nacalai Tesque, Inc.). The homogenate was centrifuged at $100000\times g$ at 4°C for 20 min. The supernatant was collected as a soluble fraction and used for the measurement of A β , BDNF, phosphorylated tau, interleukin (IL)-1 β and acetylcholine. The pellet was sonicated in 70% formic acid (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), neutralized with 20 volumes of 0.9 M Tris buffer (pH 11.0), and collected as an insoluble fraction, which was used for the measurement of insoluble A β .

Enzyme-Linked Immunosorbent Assay (ELISA) for A β , BDNF and IL-1 β The levels of A β_{1-42} and A β_{1-40} in the brain were measured using human A β ELISA kits (FUJIFILM Wako Pure Chemical Corporation) according to the manufacturer's protocol. Each soluble fraction was diluted 10-fold with the dilution buffer provided with the kit. Each insoluble fraction was diluted 1000-fold with the same buffer. The level of BDNF was measured using BDNF Emax[®] ImmunoAssay Systems (Promega Corporation, Madison, WI, U.S.A.). Each soluble fraction was diluted 3-fold with the dilution buffer provided with the kit. The level of IL-1 β was measured using Quantikine[®] ELISA kit for IL-1 β (R&D Systems, Minneapolis, MN, U.S.A.). Each soluble fraction was diluted 2-fold with the dilution buffer provided with the kit. A microplate reader Model 680 (Bio-Rad Laboratories, Hercules, CA, U.S.A.) was used to measure absorbance at 450 nm.

Western Blotting for Phosphorylated tau Measurement The level of phosphorylated tau in the brain was measured by Western blotting. Each soluble fraction was mixed with an equal amount of Tris-sodium dodecyl sulfate (SDS) β ME sample buffer (Cosmo Bio Co., Ltd., Tokyo, Japan) and boiled at 100°C for 10 min. Then, 10 μL of each sample was electrophoresed at 200 V for 1 h on a 5–20% polyacrylamide gel (FUJIFILM Wako Pure Chemical Corporation) and transferred to a 0.45 μm polyvinylidene difluoride (PVDF) membrane (Merck Millipore, Billerica, MA, U.S.A.), which was run at 15 V for 30 min. After blocking with 2.5% skimmed

Fig. 2. Spontaneous Alternation Frequency in Y-Maze Tests on Different Treatment Groups of APPswe/PS1dE9 Transgenic Mice

(A) Comparison of the control, two- and three-ingredient groups. FA + PS: 0.05% FA and 0.05% PS. FA + PS + Cur: 0.05% FA, 0.05% PS and 0.01% Cur. $N=6$ or 7 . Data are shown as mean $\pm$ S.E.M. $**p<0.01$ (Bonferroni posttest). (B) Comparison of control and Cur (0.2%) groups. $N=12$. Data are shown as mean $\pm$ S.E.M. FA, ferulic acid; PS, phosphatidylserine; Cur, curcumin; S.E.M., standard error of the mean. (C) Comparison of control and FA (0.2%) on PS (0.2%) groups. $N=5$. Data are shown as mean $\pm$ S.E. (D) The number of total arm entry of (A). $N=6$ or 7 . Data are shown as mean $\pm$ S.E.M. FA, ferulic acid; PS, phosphatidylserine; Cur, curcumin; S.E.M., standard error of the mean.

milk (Nacalai Tesque, Inc.) in Tris Buffered Saline with 0.05% Tween 20 (TBS-T) (Sigma-Aldrich Corp.) for 1 h, the blots were incubated with anti-phosphorylated (Ser202, Thr205) tau antibody AT8 (1:1000 dilution; Thermo Fisher Scientific, Waltham, MA, U.S.A.), anti-total tau antibody TAU-5 (1:2000 dilution; Thermo Fisher Scientific) or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody 14C10 (Cell Signaling Technology, Inc., Danvers, MA, U.S.A.) overnight at 4°C. The blots were washed with TBS-T for 10 min three times. The blots for AT8 and TAU-5 were then incubated with horseradish peroxidase (HRP)-conjugated anti-mouse Immunoglobulin G (1:3000 dilution; GE Healthcare, Little Chalfont, Buckinghamshire, U.K.) and the blots for GAPDH were incubated with HRP-conjugated anti-rabbit Immunoglobulin G (1:3000 dilution; GE Healthcare) for 1 h at room temperature and washed with TBS-T for 10 min three times. Phosphorylated tau was detected using Chemi-Lumi One Super (Nacalai Tesque, Inc.). Total tau and GAPDH were detected using Chemi-Lumi One L (Nacalai Tesque, Inc.). The protein bands of the blots were analyzed using an image analyzer (ImageQuant LAS4000; GE Healthcare).

Measurement of Acetylcholine The level of acetylcholine in the brain was measured using Amplitude™ fluorimetric acetylcholine assay kit (AAT Bioquest Inc., Sunnyvale, CA, U.S.A.) and a multilabel counter ALVO™ MX (PerkinElmer, Inc., Waltham, MA, U.S.A.) according to the manufacture's protocol. Each sample was diluted two-fold with the dilution

buffer provided with the kit.

Immunohistochemistry (IHC) for A β Plaque The left brain hemispheres were fixed in 4% paraformaldehyde phosphate buffer solution (FUJIFILM Wako Pure Chemical Corporation). The fixed tissues were dehydrated with ethanol (FUJIFILM Wako Pure Chemical Corporation) for 24 h, transferred into xylene (10 min three times), and embedded in paraffin (30 min three times at 60°C). The paraffin-embedded tissue blocks were cut into 5- μ m thick sections with a sliding microtome (Nippon Optical Works Co., Ltd., Tokyo, Japan) and extended on Matsunami-Adhesive Silane-coated micro slide glasses (Matsunami Glass Industry, Ltd., Osaka, Japan) at 60°C for 24 h. The sections were deparaffinized with Lemsol (FUJIFILM Wako Pure Chemical Corporation) and ethanol (100, 90, and 70%). After washing twice in water, 70% formic acid solution was dispensed on to the sections, and they were incubated at room temperature for 5 min. The sections were washed with water (5 min three times) and 0.2% Tween 20 in phosphate buffered saline (PBS) (10 min), and then stained with anti-A β antibody 6E10 (1:100 dilution; Covance Inc., Princeton, NJ, U.S.A.) using a M. O. M.™ Immunodetection Peroxidase Kit (Vector Laboratories Inc., Burlingame, CA, U.S.A.), according to the manufacture's protocol. As a chromogen, 3,3'-diaminobenzidine (FUJIFILM Wako Pure Chemical Corporation) at a concentration of 0.5 mg/mL in PBS with 0.005% hydrogen peroxide was used. After staining, the sections were washed with water, dehydrated with ethanol (70,

Fig. 3. Comparison of Biological Brain Parameters for the Control, Two-Ingredient and Three-Ingredient Groups

(A) Insoluble $A\beta_{1-42}$, (B) insoluble $A\beta_{1-40}$, (C) soluble $A\beta_{1-42}$, (D) soluble $A\beta_{1-40}$, (E) BDNF, (F) IL-1 β , (G) phosphorylated tau (AT8) normalized by GAPDH, (H) total tau (TAU-5) normalized by GAPDH, (I) phosphorylated tau–total tau ratio, (J) representative images of tau Western blotting, and (K) acetylcholine. $N=6$ or 7. Data are shown as mean $\pm$ S.E.M.; * $p<0.05$ and ** $p<0.01$ vs. the control group (Dunnett test or Mann–Whitney U test). $A\beta$, amyloid- β ; BDNF, brain-derived neurotrophic factor; IL-1 β , interleukin 1 beta; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; S.E.M., standard error of the mean.

90, and 100%) and Lemosol, and then mounted with mounting medium (Daido Sangyo Co., Ltd., Tokyo, Japan). The sections were observed with a microscope BZ-8100 (Keyence Corp., Osaka, Japan). The ratio of the area of $A\beta$ plaques to that of the whole section was calculated using ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A.).

Data Analysis Each data was expressed as mean $\pm$ standard error of the mean (S.E.M.). Data derived from the Y-maze test were analyzed using two-way ANOVA and the Bonferroni posttest. Data derived from biochemical tests and IHC were analyzed using one-way ANOVA and the Dunnett's posttest or the Mann–Whitney U test. The software Graphpad Prism (GraphPad Software Inc., San Diego, CA, U.S.A.) was used for these analyses, and $p<0.05$ was considered statistically significant.

RESULTS

Spatial working memories of APPswe/PS1dE9 transgenic

mice were evaluated using Y-maze tests during treatment with two ingredients (FA and PS) or three ingredients (FA, PS, and Cur) (Fig. 2A). At the start of treatment (7-month-old mice), the spontaneous alternation frequency of each group was about approx. 60–63: $60.9 \pm 4.6\%$ for the control group, $62.2 \pm 3.3\%$ for the two-ingredient group, and $60.6 \pm 3.9\%$ for the three-ingredient group. Over 3 months of treatment, the spontaneous alternation frequency of the control group significantly decreased to $42.4 \pm 8.8\%$, while that of the two- and three-ingredient groups did not decrease much: $54.7 \pm 7.1\%$ and $58.3 \pm 5.5\%$, respectively. The difference between the three-ingredient group and the control group was significant ($p=0.031$ by two-way ANOVA and $p<0.01$ at week 12 by the Bonferroni posttest). For comparison, we evaluated the efficacy of Cur alone (Fig. 2B). At the start of treatment, the spontaneous alternation frequency was $60.1 \pm 2.2\%$ for the control group and $59.9 \pm 2.2\%$ for the Cur group. After 3 months of treatment, the spontaneous alternation frequency of the control and Cur groups decreased to $49.4 \pm 2.7\%$ and

Fig. 4. Comparison of Biological Brain Parameters for the Control and Cur Groups

(A) Insoluble A β_{1-42} , (B) insoluble A β_{1-40} , (C) soluble A β_{1-42} , (D) soluble A β_{1-40} , (E) BDNF, (F) IL-1 β , (G) phosphorylated tau (AT8) normalized by GAPDH, (H) total tau (TAU-5) normalized by GAPDH, (I) phosphorylated tau-total tau ratio, (J) representative images of tau Western blotting, and (K) acetylcholine. $N = 12$. Data are shown as mean $\pm$ S.E.M.; * $p < 0.05$ vs. the control group (Mann-Whitney U test). Cur, curcumin; A β , amyloid- β ; BDNF, brain-derived neurotrophic factor; IL-1 β , interleukin 1 beta; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; S.E.M., standard error of the mean.

49.6 $\pm$ 2.6%, respectively. Furthermore, we also confirmed the efficacy of FA or PS alone (Fig. 2C). At the start of treatment, spontaneous alternation frequency was 60.1 $\pm$ 3.4% for the control group, 61.0 $\pm$ 4.4% for the FA group and 60.7 $\pm$ 7.1% for the PS group. After 3 months of treatment, the spontaneous alternation frequency of these groups was decreased to 45.9 $\pm$ 9.6%, 46.5 $\pm$ 7.9%, and 46.5 $\pm$ 6.3%, respectively. These results indicated that the combined use of FA, PS, and Cur has additive and synergistic therapeutic or preventive effects on cognitive dysfunction.

We observed a tendency of a decrease in the number of total arm entries in all the groups at week 12 of treatment (Fig. 2D), indicating that an age-related decline in motor function might occur in the mice in this study. Therefore, we did not conduct other memory tests.

After 3 months of treatment, we determined the amounts of A β , BDNF, IL-1 β , acetylcholine, and phosphorylated tau in the mouse brains (Figs. 3, 4). Insoluble A β_{1-42} (Fig. 3A) and insoluble A β_{1-40} (Fig. 3B) significantly decreased in the

three-ingredient group compared to the control group (A β_{1-42} : $p = 0.019$ by one-way ANOVA and $p < 0.05$ by Dunnett test; A β_{1-40} : $p = 0.014$ by one-way ANOVA and $p < 0.01$ by the Dunnett test). There was also a slight decrease in the soluble A β in the two- and three-ingredient groups, but no significant difference was observed (Figs. 3C, D). In addition, in the Cur group, A β decreased, and there was a significant difference in soluble A β_{1-40} compared with the control group ($p = 0.038$ by the Mann-Whitney U test; Fig. 4D). BDNF was slightly higher in the three-ingredient group compared to the control group, but the difference was not statistically significant ($p = 0.77$ by one-way ANOVA; Fig. 3E). In addition, BDNF was slightly higher in the Cur group compared to the control group, but again, the difference was not significant ($p = 0.22$ by the Mann-Whitney U test; Fig. 4E). IL-1 β , which is an inflammatory cytokine, decreased in the two- and three-ingredient groups ($p = 0.10$ by one-way ANOVA; Fig. 3F) and was significantly lower in both groups compared to the control group ($p = 0.036$ and $p = 0.023$ by the Mann-Whitney U test). In

Fig. 5. Immunohistochemical Analysis of Brain A β in the Control Group and the Three-Ingredient Group

Brain sections were immunostained with anti-A β antibody 6E10. Percentage of the area of A β plaques to the total area of brain sections. $N=6$. Data are shown as mean $\pm$ S.E.M. Representative photos of each treatment group. Scale bar = 1 mm. A β , amyloid- β ; S.E.M., standard error of the mean.

addition, IL-1 β was slightly lower in the Cur group compared to the control group, but the difference was not significant ($p=0.18$ by the Mann-Whitney U test; Fig. 4F). Phosphorylated tau detected by AT8 normalized by GAPDH increased in the two- and three-ingredient groups compared to the control group ($p=0.045$ by one-way ANOVA and $p<0.05$ by the Dunnett test; Fig. 3G). Interestingly, we found no change in phosphorylated tau between the two-ingredient group and the control group, and the total tau detected by TAU-5 significantly increased in the three-ingredient group (Fig. 3H). As a result, the AT8/TAU-5 ratio was significantly lower in the three-ingredient group compared to the control group ($p=0.045$ by one-way ANOVA and $p<0.05$ by the Dunnett test; Fig. 3I). In contrast, phosphorylated tau decreased in the Cur group compared to the control group ($p=0.12$ by one-way ANOVA), and there was a significant difference in the AT8/TAU-5 ratio for the Cur and control groups ($p=0.049$ by the Mann-Whitney U test; Fig. 4I). Acetylcholine slightly increased in the two- and three-ingredient groups compared to the control group but not significantly ($p=0.12$ by one-way ANOVA; Fig. 3K). In addition, there was no significant difference in the acetylcholine levels in the Cur and control groups ($p=0.49$, Mann-Whitney U test; Fig. 4K).

Brain sections from the control group and the three-ingredient group that showed a significant difference in insoluble A β s were immunostained with anti-A β antibody 6E10 (Fig. 5). The area of A β plaques decreased by 29% in the three-ingredient group compared with the control group. This result agreed with the results of ELISA for insoluble A β , but there was no significant difference between the groups ($p=0.09$ by the Mann-Whitney U test).

DISCUSSION

In AD, A β abnormalities precede the onset of cognitive dysfunction by approximately 20 years,⁵¹⁾ so it is too late to administer drugs and/or other therapies after cognitive dysfunction onset. In addition, the currently approved AD drugs, such as AChE inhibitors and NMDA receptor antagonist, are only symptomatic treatments. Realistic measures for AD treatment would include long-term preventive approaches. One such effective long-term approach is the consumption of active ingredients every day. However, spoiling the taste and smell of food by using large amounts of biological ingredients is not desirable. Even when they are taken as dietary supplements, it might be difficult to maintain large daily doses. Therefore, we reasoned that additive and synergistic effects of combining multiple active ingredients, even at low doses, might overcome these difficulties.

In this study, we chose FA, PS and Cur as active ingredients because each has a different mechanism of action (MOA) and their combination might directly improve multiplex AD-specific pathologies. To begin this study, we compared a few ingredients that are considered good for cognitive function in terms of their MOAs (Table 1). As mentioned before, FA inhibits A β production *via* down-regulation of APP and β -secretase,⁶⁾ inhibits A β aggregation,⁸⁾ and protects nerve cells from A β -induced neurotoxicity,¹²⁾ but few studies have reported improvement of tau pathology, except promotion of tau dephosphorylation⁹⁾ and phosphorylated tau clearance.¹⁰⁾ Therefore, FA is expected to act relatively upstream of the pathophysiologic mechanism of AD. On the other hand, many studies have reported that Cur not only inhibits A β production^{28,25)} but also inhibits tau phosphorylation^{32,39,40)} and its aggregation.^{41,42)} Therefore, Cur is also expected to act downstream of the pathophysiologic mechanism of AD. In addition, FA reportedly activates acetylcholine receptor,¹³⁾ but Cur does not. On the other hand, Cur reportedly inhibits AChE activity,³¹⁾ but FA does not. On the basis of these findings, we believe that FA and Cur might complement each other.

We also compared a few polyphenols besides FA and Cur. Catechin and its analogs are flavonoid polyphenols that are abundant in green tea. Among them, epigallocatechin gallate (EGCG) reportedly inhibits A β production *via* α -secretase activation⁵²⁾ and β -secretase inhibition,⁵³⁾ inhibits A β aggregation,⁵⁴⁾ promotes A β ⁵⁵⁾ and phosphorylated tau⁵⁶⁾ clearance *via* autophagy,⁵⁵⁾ inhibits tau aggregation,⁵⁷⁾ protects neural cells from oxidative stress and A β -induced neurotoxicity,^{58,59)} inhibits AChE activity,⁶⁰⁾ and promotes neurogenesis.^{61,62)} In addition, more than 10 clinical trials have been conducted on EGCG or green tea,⁶³⁾ and some have shown efficacy in improving cognitive function. However, EGCG also promotes tau phosphorylation *via* GSK3 β up-regulation⁶⁴⁾ and AChE activation,⁶⁵⁾ which might worsen AD. Therefore, we did not select EGCG and other catechins in this study.

Other flavonoid polyphenols, such as quercetin, myricetin, and taxifolin, also reportedly are effective against AD. Quercetin inhibits A β production *via* α -secretase up-regulation⁶⁶⁾ and β -secretase⁶⁷⁾ and γ -secretase inhibition,⁶⁸⁾ inhibits A β aggregation,⁶⁹⁾ promotes A β clearance,⁷⁰⁾ protects nerve cells from A β -induced cytotoxicity,^{71,72)} and activates the CREB/BDNF pathway.⁷³⁾ Myricetin inhibits A β production *via*

Table 1. Comparison of MOAs of Some Active Ingredients Related to AD Prevention and Treatment

Mechanism of action	The ingredients in the present study				Polyphenols				Polyunsaturated fatty acids		Phospholipids	
	FA	PS	Cur	Catechin	Quercetin	Myricetin	Taxifolin	Resveratrol	DHA, EPA	α -Linolenic acid	Phosphatidylcholine	Plasmalogen
$A\beta$ generation inhibition	6		28									
$A\beta$ aggregation inhibition $A\beta$ clearance promotion												
Tau phosphorylation inhibition												
Tau aggregation inhibition Tau clearance promotion Neuroprotection												
Neurotransmission												
Neurogenesis												

MOAs, mechanisms of action; AD, Alzheimer's disease.

α -secretase activation and β -secretase inhibition,⁷⁴⁾ inhibits A β aggregation,^{8,75)} and promotes an increase in BDNF.⁷⁶⁾

Taxifolin inhibits A β production via β -secretase up-regulation,⁷⁷⁾ inhibits A β aggregation,^{78,79)} and inhibits AChE activity.⁸⁰⁾ Although a clinical trial on a quercetin-rich onion powder was conducted,⁸¹⁾ it was difficult to judge the effectiveness of the powder because the scale of the trial was very small ($N=4$ to 5 people). In addition, the reported MOA almost overlapped with the MOAs of FA and Cur. Therefore, these ingredients were excluded from this study.

Resveratrol, a stilbenoid polyphenol, is known as a sirtuin activator.⁸²⁾ Sirtuin is involved in α -secretase activation⁸³⁾ and tau aggregation inhibition.⁸⁴⁾ In fact, studies have reported that resveratrol reduces A β via α -secretase activation.⁸⁵⁾ Sirtuin also has neuroprotective effects,⁸⁶⁾ and resveratrol reportedly protects neural cells from A β -induced neurotoxicity via activation of the sirtuin pathway.⁸⁷⁾ Resveratrol also inhibits A β production via β -secretase inhibition,⁸⁸⁾ inhibits A β aggregation,⁸⁹⁾ promotes A β clearance,^{90,91)} reduces phosphorylated tau via GSK3 inhibition,⁹²⁾ protects nerve cells from A β -induced cytotoxicity,^{93,94)} inhibits AChE activity,⁹⁵⁾ regulates proper expression of the NMDA receptor,⁹⁶⁾ and promotes an increase in BDNF.⁹⁷⁾ However, a clinical trial on resveratrol for AD treatment showed no positive effect⁹⁸⁾; in fact, resveratrol treatment seemed to worsen the brain condition compared with the placebo, so we did not include it in this study.

Polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) and omega-3 eicosapentaenoic acid (EPA) from fish oil, can also be effective against AD. Polyunsaturated fatty acids are important components of the cell membrane and contribute to proper nerve cell function. In fact, in an AD patient's brain, DHA decreases.⁹⁹⁾ Therefore, the dietary intake of these polyunsaturated fatty acids could be effective in preventing and treating AD. DHA reportedly inhibits A β production via β -secretase inhibition¹⁰⁰⁾ and γ -secretase down-regulation,¹⁰¹⁾ inhibits A β aggregation,¹⁰²⁾ promotes A β clearance,^{103,104)} protects neural cells from oxidative stress,^{105–107)} promotes acetylcholine release,¹⁰⁸⁾ regulates the NMDA receptor,¹⁰⁹⁾ and promotes neurogenesis.¹¹⁰⁾ Several clinical trials on DHA/EPA (as fish oil) for AD patients have been conducted, with some of them showing positive results.¹¹¹⁾

In addition, α -linolenic acid, which is a precursor of DHA/EPA and rich in linseed oil, also reportedly has anti-AD actions such as α -secretase up-regulation,¹⁰³⁾ β -secretase inhibition,^{112,113)} γ -secretase inhibition,¹¹⁴⁾ neuroprotection,^{115,116)} acetylcholine receptor regulation,¹¹⁷⁾ and promotion of BDNF expression.¹¹⁸⁾ However, α -linolenic acid, DHA, and EPA are easily susceptible to oxidation. Not only does oxidized fish oil show no positive effects,^{119,120)} but it may also have negative effects on health.¹²¹⁾ We believed it was difficult to prevent oxidation of polyunsaturated fatty acids when mixed with food, so these were excluded from this study.

Some kinds of phospholipids, such as phosphatidylcholine (PC; also called lecithin) or plasmalogens other than PS, also seem effective in preventing and treating AD. PC, like PS,^{22–24,122)} is an important factor that makes up the nerve cell membrane and also a source of acetylcholine.^{123,124)} PC is found to decrease in an AD patient's brain,⁹⁹⁾ so replenishment of PC is expected to prevent and treat AD. PC reportedly protects nerve cells from A β -induced neurotoxicity.¹²⁵⁾ More than 10 clinical trials on PC for AD treatment have

been conducted, but no trials showed a clear improvement in cognitive function.¹²⁶⁾ Plasmalogen also decreases in an AD patient's brain,¹²⁷⁾ and the brain level of plasmalogen is correlated with cognitive function in AD patients.¹²⁸⁾ A clinical trial on plasmalogen for mild AD treatment showed improvement in cognitive function.¹²⁹⁾ Plasmalogen also inhibits γ -secretase,¹³⁰⁾ provides neuroprotection from oxidative stress,¹³¹⁾ and increases acetylcholine receptor levels.¹³²⁾ It is believed that the actions and functions of phospholipids cannot be replaced by polyphenols, so one phospholipid should be added to FA and Cur. Many studies have reported the effectiveness of PS against AD. Therefore, we selected PS and expected that the combined use of FA, PS, and Cur would result in a higher therapeutic effect on cognitive dysfunction in the AD mice model compared with each ingredient alone.

In fact, oral administration of the three ingredients in APPswe/PS1dE9 transgenic mice resulted in significant improvements in cognitive dysfunction, as evaluated by Y-maze tests (Fig. 2A), but did not each alone (Figs. 2B, C). A β was significantly lower in the three-ingredient group (Figs. 3A, 3B) and the Cur group (Figs. 4A, B) compared with the control group. In addition, in the two-ingredient group, A β showed a slightly decreasing trend indicating that both FA and Cur contribute to A β reduction. BDNF increased in the three-ingredient group and the Cur group (Figs. 3E, 4E), suggesting that Cur is involved in BDNF increase.

In addition, in the ingredient-treatment groups, tau expression and phosphorylation seemed to change compared with the control group (Figs. 3G–I, 4G–I). Interestingly, in the three-ingredient group, tau phosphorylation significantly decreased (Fig. 3I) because phosphorylated tau did not decrease (Fig. 3G), but total tau drastically increased (Fig. 3H). In the groups of control and two-ingredient, total tau might decrease because of neuronal loss and Cur might attenuate it. In addition, in the Cur group, tau phosphorylation significantly decreased (Fig. 4I). However, phosphorylated tau tended to decrease (Fig. 4G), and total tau did not increase so much (Fig. 4H). These results of Cur were consistent with previous studies—many studies have reported that Cur has inhibitory effects on tau phosphorylation compared with FA and PS.

Acetylcholine was increased in the two- and three-ingredient groups (Fig. 3K). This increasing effect on acetylcholine was believed to be due to PS, as reported previously.^{22–24)} However, we did not observe any increase in acetylcholine in the Cur group (Fig. 4K). Cur reportedly shows AChE inhibitory activity,³³⁾ but we could not confirm it this study.

IL-1 β decreased in the two- and three-ingredient groups (Fig. 3F). This effect is believed to be due to FA, because there was no difference in the amounts of IL-1 β between the two- and three-ingredient groups (Fig. 3F) and between the Cur and control groups (Fig. 4F). Studies have reported that Cur down-regulates IL-1 β expression,¹³³⁾ but we could not confirm it in this study.

In this study, only the three-ingredient group exhibited a significant improvement in cognitive function despite a significant decrease in A β , IL-1 β , or phosphorylated tau in other groups. These results indicated that the cognitive function of the APPswe/PS1dE9 transgenic mice improved by using multiple substances with different effective MOAs. In fact, studies have reported that the combined use of FA and other polyphenols ameliorates cognitive dysfunction and AD-like

pathology in mice.^{134,135} Therefore, such combined use might be an effective AD prevention or treatment strategy, not only in mice models but also for humans.

Acknowledgments The authors would like to thank Dr. Katsuya Urakami (Tottori University) for helpful advices on a study design and a draft of the manuscript.

Conflict of Interest H. Sugimoto is President of Green Tech Co., Ltd. and M. Okuda and Y. Fujita are employees of it.

REFERENCES

- Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. *EMBO J.*, **4**, 2757–2763 (1985).
- Wischik CM, Novak M, Thøgersen HC, Edwards PC, Runswick MJ, Jakes R, Walker JE, Milstein C, Roth M, Klug A. Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 4506–4510 (1988).
- Choi DY, Lee YJ, Hong JT, Lee HJ. Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. *Brain Res. Bull.*, **87**, 144–153 (2012).
- Gómez-Pinilla F. Brain foods: the effects of nutrients on brain function. *Nat. Rev. Neurosci.*, **9**, 568–578 (2008).
- Ou S, Kwok K-C. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *J. Sci. Food Agric.*, **84**, 1261–1269 (2004).
- Nagai N, Kotani S, Mano Y, Ueno A, Ito Y, Kitaba T, Takata T, Fujii N. Ferulic acid suppresses amyloid β production in the human lens epithelial cell stimulated with hydrogen peroxide. *Biomed Res Int.*, **2017**, 5343010 (2017).
- Mori T, Koyama N, Guillot-Sestier MV, Tan J, Town T. Ferulic acid is a nutraceutical β -secretase modulator that improves behavioral impairment and alzheimer-like pathology in transgenic mice. *PLOS ONE*, **8**, e55774 (2013).
- Hamaguchi T, Ono K, Murase A, Yamada M. Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. *Am. J. Pathol.*, **175**, 2557–2565 (2009).
- Koh PO. Ferulic acid attenuates the injury-induced decrease of protein phosphatase 2A subunit B in ischemic brain injury. *PLOS ONE*, **8**, e54217 (2013).
- Bian Z, Furuya N, Zheng DM, Oliva Trejo JA, Tada N, Ezaki J, Ueno T. Ferulic acid induces mammalian target of rapamycin inactivation in cultured mammalian cells. *Biol. Pharm. Bull.*, **36**, 120–124 (2013).
- Barone E, Calabrese V, Mancuso C. Ferulic acid and its therapeutic potential as a hormetin for age-related diseases. *Biogerontology*, **10**, 97–108 (2009).
- Kim HS, Cho JY, Kim DH, Yan JJ, Lee HK, Suh HW, Song DK. Inhibitory effects of long term administration of ferulic acid on microglial activation induced by intercerebroventricular injection of beta amyloid peptide (1–42) in mice. *Biol. Pharm. Bull.*, **27**, 120–121 (2004).
- Tsai FS, Wu LY, Yang SE, Cheng HY, Tsai CC, Wu CR, Lin LW. Ferulic acid reverses the cognitive dysfunction caused by amyloid β peptide 1–40 through anti-oxidant activity and cholinergic activation in rats. *Am. J. Chin. Med.*, **43**, 319–335 (2015).
- Yabe T, Hirahara H, Harada N, Ito N, Nagai T, Sanagi T, Yamada H. Ferulic acid induces neural progenitor cell proliferation *in vitro* and *in vivo*. *Neuroscience*, **165**, 515–524 (2010).
- van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.*, **9**, 112–124 (2008).
- Takai Y, Kishimoto A, Iwasa Y, Kawahara Y, Mori T, Nishizuka Y. Calcium dependent activation of a multifunctional protein kinase by membrane phospholipids. *J. Biol. Chem.*, **254**, 3692–3695 (1979).
- Wrenn RW, Katoh N, Wise BC, Kuo JF. Stimulation by phosphatidylserine and calmodulin of calcium-dependent phosphorylation of endogenous proteins from cerebral cortex. *J. Biol. Chem.*, **255**, 12042–12046 (1980).
- Tsakiris S, Deliconstantinos G. Influence of phosphatidylserine on (Na⁺ + K⁺)-stimulated ATPase and acetylcholinesterase activities of dog brain synaptosomal plasma membranes. *Biochem. J.*, **220**, 301–307 (1984).
- Cohen SA, Muller WE. Age-related alterations of NMDA-receptor properties in the mouse forebrain: partial restoration by chronic phosphatidylserine treatment. *Brain Res.*, **584**, 174–180 (1992).
- Crook TH, Tinklenberg J, Yesavage J, Petrie W, Nunzi MG, Masari DC. Effects of phosphatidylserine in age-associated memory impairment. *Neurology*, **41**, 644–649 (1991).
- Crook T, Petrie W, Wells C, Massari DC. Effects of phosphatidylserine in Alzheimer's disease. *Psychopharmacol. Bull.*, **28**, 61–66 (1992).
- Casamenti F, Scali C, Pepeu G. Phosphatidylserine reverses the age dependent decrease in cortical acetylcholine release: a microdialysis study. *Eur. J. Pharmacol.*, **194**, 11–16 (1991).
- Rosadini G, Sannita WG, Nobili F, Cenacchi T. Phosphatidylserine: quantitative EEG effects in healthy volunteers. *Neuropsychobiology*, **24**, 42–48 (1990–1991).
- Pedata F, Giovannelli L, Spignoli G, Giovannini MG, Pepeu G. 1985 Phosphatidylserine increases acetylcholine release from cortical slices in aged rats. *Neurobiol. Aging*, **6**, 337–339 (1985).
- Zhang YY, Yang LQ, Guo LM. Effect of phosphatidylserine on memory in patients and rats with Alzheimer's disease. *Genet. Mol. Res.*, **14**, 9325–9333 (2015).
- Cohen SA, Müller WE. Age-related alterations of NMDA-receptor properties in the mouse forebrain: partial restoration by chronic phosphatidylserine treatment. *Brain Res.*, **584**, 174–180 (1992).
- Yuyama K, Sun H, Mitsutake S, Igarashi Y. Sphingolipid-modulated exosome secretion promotes clearance of amyloid- β by microglia. *J. Biol. Chem.*, **287**, 10977–10989 (2012).
- Zhang C, Browne A, Child D, Tanzi RE. Curcumin decreases amyloid-beta peptide levels by attenuating the maturation of amyloid-beta precursor protein. *J. Biol. Chem.*, **285**, 28472–28480 (2010).
- Narasingappa RB, Javagal MR, Pullabhatla S, Htoo HH, Rao JK, Hernandez JF, Govitrapong P, Vincent B. Activation of α -secretase by curcumin-amino acid conjugates. *Biochem. Biophys. Res. Commun.*, **424**, 691–696 (2012).
- Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H. Epigallocatechin-3-gallate and curcumin suppress amyloid beta-induced beta-site APP cleaving enzyme-1 upregulation. *Neuroreport*, **19**, 1329–1333 (2008).
- Murata K, Matsumura S, Yoshioka Y, Ueno Y, Matsuda H. Screening of β -secretase and acetylcholinesterase inhibitors from plant resources. *J. Nat. Med.*, **69**, 123–129 (2015).
- Di Martino RM, De Simone A, Andrisano V, Bisignano P, Bisi A, Gobbi S, Rampa A, Fato R, Bergamini C, Perez DI, Martinez A, Bottegoni G, Cavalli A, Belluti F. Versatility of the Curcumin scaffold: discovery of potent and balanced dual BACE-1 and GSK-3 β inhibitors. *J. Med. Chem.*, **59**, 531–544 (2016).
- Yoshida H, Okumura N, Nishimura Y, Kitagishi Y, Matsuda S. Turmeric and curcumin suppress presenilin 1 protein expression in Jurkat cells. *Exp. Ther. Med.*, **2**, 629–632 (2011).
- Narlawar R, Baumann K, Schubel R, Schmidt B. Curcumin

- derivatives inhibit or modulate beta-amyloid precursor protein metabolism. *Neurodegener. Dis.*, **4**, 88–93 (2007).
- 35) Kotani R, Urano Y, Sugimoto H, Noguchi N. Decrease of amyloid- β levels by curcumin derivative *via* modulation of amyloid- β protein precursor trafficking. *J. Alzheimer's Dis.*, **56**, 529–542 (2017).
 - 36) Yang F, Lim GP, Begum AN, Ubada OJ, Simmons MR, Ambegaokar SS, Chen P, Kaye R, Glabe CG, Frautschy SA, Cole GM. Curcumin inhibits formation of amyloid β oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. *J. Biol. Chem.*, **280**, 5892–5901 (2005).
 - 37) Wang P, Su C, Li R, Wang H, Ren Y, Sun H, Yang J, Sun J, Shi J, Tian J, Jiang S. Mechanisms and effects of curcumin on spatial learning and memory improvement in APPswe/PS1dE9 mice. *J. Neurosci. Res.*, **92**, 218–231 (2014).
 - 38) Shinojima N, Yokoyama T, Kondo Y, Kondo S. Roles of the Akt/mTOR/p70S6K and ERK1/2 signaling pathways in curcumin-induced autophagy. *Autophagy*, **3**, 635–637 (2007).
 - 39) Zhang X, Yin WK, Shi XD, Li Y. Curcumin activates Wnt/ β -catenin signaling pathway through inhibiting the activity of GSK-3 β in APPswe transfected SY5Y cells. *Eur. J. Pharm. Sci.*, **42**, 540–546 (2011).
 - 40) Sundaram JR, Poore CP, Sulaimanee NHB, Pareek T, Cheong WF, Wenk MR, Pant HC, Frautschy SA, Low CM, Kesavapany S. Curcumin ameliorates neuroinflammation, neurodegeneration, and memory deficits in p25 transgenic mouse model that bears hallmarks of Alzheimer's disease. *J. Alzheimer's Dis.*, **60**, 1429–1442 (2017).
 - 41) Dolai S, Shi W, Corbo C, Sun C, Averick S, Obeysekera D, Farid M, Alonso A, Banerjee P, Raja K. “Clicked” sugar–curcumin conjugate: modulator of amyloid- β and tau peptide aggregation at ultralow concentrations. *ACS Chem. Neurosci.*, **2**, 694–699 (2011).
 - 42) Ma QL, Zuo X, Yang F, Ubada OJ, Gant DJ, Alaverdyan M, Teng E, Hu S, Chen PP, Maiti P, Teter B, Cole GM, Frautschy SA. Curcumin suppresses soluble Tau dimers and corrects molecular chaperone, synaptic, and behavioral deficits in aged human Tau transgenic mice. *J. Biol. Chem.*, **288**, 4056–4065 (2013).
 - 43) Kim HY, Park EJ, Joe EH, Jou I. Curcumin suppresses Janus kinase-STAT inflammatory signaling through activation of Src homology 2 domain-containing tyrosine phosphatase 2 in brain microglia. *J. Immunol.*, **171**, 6072–6079 (2003).
 - 44) Park SY, Kim HS, Cho EK, Kwon BY, Phark S, Hwang KW, Sul D. Curcumin protected PC12 cells against beta-amyloid-induced toxicity through the inhibition of oxidative damage and tau hyperphosphorylation. *Food Chem. Toxicol.*, **46**, 2881–2887 (2008).
 - 45) Chen RW, Qin ZH, Ren M, Kanai H, Chalecka-Franaszek E, Leeds P, Chuang DM. Regulation of c-Jun N-terminal kinase, p38 kinase and AP-1 DNA binding in cultured brain neurons: roles in glutamate excitotoxicity and lithium neuroprotection. *J. Neurochem.*, **84**, 566–575 (2003).
 - 46) Kang SK, Cha SH, Jeon HG. Curcumin-induced histone hypoacetylation enhances caspase-3-dependent glioma cell death and neurogenesis of neural progenitor cells. *Stem Cells Dev.*, **15**, 165–174 (2006).
 - 47) Xu Y, Ku B, Cui L, Li X, Barish PA, Foster TC, Ogle WO. Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. *Brain Res.*, **1162**, 9–18 (2007).
 - 48) Chandra V, Pandav R, Dodge HH, Johnston JM, Belle SH, DeKosky ST, Ganguli M. Incidence of Alzheimer's disease in a rural community in India: the Indo–U.S. study. *Neurology*, **57**, 985–989 (2001).
 - 49) Small GW, Siddarth P, Li Z, Miller KJ, Ercoli L, Emerson ND, Martinez J, Wong KP, Liu J, Merrill DA, Chen ST, Henning SM, Satyamurthy N, Huang SC, Heber D, Barrio JR. Memory and brain amyloid and tau effects of a bioavailable form of curcumin in non-demented adults: a double-blind, placebo-controlled 18-month trial. *Am. J. Geriatr. Psychiatry*, **26**, 266–277 (2018).
 - 50) Brondino N, Re S, Boldrini A, Cuccomaro A, Lanati N, Barale F, Politi P. Curcumin as a therapeutic agent in dementia: a mini systematic review of human studies. *ScientificWorldJournal*, **2014**, 174282 (2014).
 - 51) Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.*, **9**, 119–128 (2010).
 - 52) Levites Y, Amit T, Mandel S, Youdim MB. Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (–)-epigallocatechin-3-gallate. *FASEB J.*, **17**, 952–954 (2003).
 - 53) Jeon SY, Bae K, Seong YH, Song KS. Green tea catechins as a BACE1 (beta-secretase) inhibitor. *Bioorg. Med. Chem. Lett.*, **13**, 3905–3908 (2003).
 - 54) Ehrnhoefer DE, Bieschke J, Boeddrich A, Herbst M, Masino L, Lurz R, Engemann S, Pastore A, Wanker EE. EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. *Nat. Struct. Mol. Biol.*, **15**, 558–566 (2008).
 - 55) Gu HF, Nie YX, Tong QZ, Tang YL, Zeng Y, Jing KQ, Zheng XL, Liao DF. Epigallocatechin-3-gallate attenuates impairment of learning and memory in chronic unpredictable mild stress-treated rats by restoring hippocampal autophagic flux. *PLOS ONE*, **9**, e112683 (2014).
 - 56) Chesser AS, Ganeshan V, Yang J, Johnson GV. Epigallocatechin-3-gallate enhances clearance of phosphorylated tau in primary neurons. *Nutr. Neurosci.*, **19**, 21–31 (2016).
 - 57) Taniguchi S, Suzuki N, Masuda M, Hisanaga S, Iwatsubo T, Goedert M, Hasegawa M. Inhibition of heparin-induced tau filament formation by phenothiazines, polyphenols, and porphyrins. *J. Biol. Chem.*, **280**, 7614–7623 (2005).
 - 58) He Y, Cui J, Lee JC, Ding S, Chalimoniuk M, Simonyi A, Sun AY, Gu Z, Weisman GA, Wood WG, Sun GY. Prolonged exposure of cortical neurons to oligomeric amyloid- β impairs NMDA receptor function *via* NADPH oxidase-mediated ROS production: protective effect of green tea (–)-epigallocatechin-3-gallate. *ASN Neuro*, **3**, e00050 (2011).
 - 59) Levites Y, Amit T, Mandel S, Youdim MB. Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (–)-epigallocatechin-3-gallate. *FASEB J.*, **17**, 952–954 (2003).
 - 60) Srividhya R, Gayathri R, Kalaiselvi P. Impact of epigallocatechin-3-gallate on acetylcholine–acetylcholine esterase cycle in aged rat brain. *Neurochem. Int.*, **60**, 517–522 (2012).
 - 61) Li Q, Zhao HF, Zhang ZF, Liu ZG, Pei XR, Wang JB, Cai MY, Li Y. Long-term administration of green tea catechins prevents age-related spatial learning and memory decline in C57BL/6J mice by regulating hippocampal cyclic amp-response element binding protein signaling cascade. *Neuroscience*, **159**, 1208–1215 (2009).
 - 62) Wang Y, Li M, Xu X, Song M, Tao H, Bai Y. Green tea epigallocatechin-3-gallate (EGCG) promotes neural progenitor cell proliferation and sonic hedgehog pathway activation during adult hippocampal neurogenesis. *Mol. Nutr. Food Res.*, **56**, 1292–1303 (2012).
 - 63) Ide K, Matsuoka N, Yamada H, Furushima D, Kawakami K. Effects of tea catechins on Alzheimer's disease: recent updates and perspectives. *Molecules*, **23**, E2357 (2018).
 - 64) Miyai S, Yamaguchi A, Iwasaki T, Shamsa F, Ohtsuki K. Biochemical characterization of epigallocatechin-3-gallate as an effective stimulator for the phosphorylation of its binding proteins by glycogen synthase kinase-3 β *in vitro*. *Biol. Pharm. Bull.*, **33**, 1932–1937 (2010).
 - 65) Srividhya R, Gayathri R, Kalaiselvi P. Impact of epigallocatechin-

- 3-gallate on acetylcholine-acetylcholine esterase cycle in aged rat brain. *Neurochem. Int.*, **60**, 517–522 (2012).
- 66) Martín-Aragón S, Jiménez-Aliaga KL, Benedí J, Bermejo-Bescós P. Neurohormetic responses of quercetin and rutin in a cell line over-expressing the amyloid precursor protein (APPswe cells). *Phytomedicine*, **23**, 1285–1294 (2016).
- 67) Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H. Flavonols and flavones as BACE-1 inhibitors: structure–activity relationship in cell-free, cell-based and *in silico* studies reveal novel pharmacophore features. *Biochim. Biophys. Acta*, **1780**, 819–825 (2008).
- 68) Ohta K, Mizuno A, Li S, Itoh M, Ueda M, Ohta E, Hida Y, Wang MX, Furoi M, Tsuzuki Y, Sobajima M, Bohmoto Y, Fukushima T, Kobori M, Inuzuka T, Nakagawa T. Endoplasmic reticulum stress enhances γ -secretase activity. *Biochem. Biophys. Res. Commun.*, **416**, 362–366 (2011).
- 69) Matsuzaki K, Noguch T, Wakabayashi M, Ikeda K, Okada T, Ohashi Y, Hoshino M, Naiki H. Inhibitors of amyloid beta-protein aggregation mediated by GM1-containing raft-like membranes. *Biochim. Biophys. Acta*, **1768**, 122–130 (2007).
- 70) Regitz C, DuBling LM, Wenzel U. Amyloid-beta ($A\beta_{1-42}$)-induced paralysis in *Caenorhabditis elegans* is inhibited by the polyphenol quercetin through activation of protein degradation pathways. *Mol. Nutr. Food Res.*, **58**, 1931–1940 (2014).
- 71) Dajas F, Rivera F, Blasina F, Arredondo F, Echeverry C, Lafon L, Morquio A, Heizen H. Cell culture protection and *in vivo* neuroprotective capacity of flavonoids. *Neurotox. Res.*, **5**, 425–432 (2003).
- 72) Zhu JT, Choi RC, Chu GK, Cheung AW, Gao QT, Li J, Jiang ZY, Dong TT, Tsim KW. Flavonoids possess neuroprotective effects on cultured pheochromocytoma PC12 cells: a comparison of different flavonoids in activating estrogenic effect and in preventing beta-amyloid-induced cell death. *J. Agric. Food Chem.*, **55**, 2438–2445 (2007).
- 73) Tchantchou F, Lacor PN, Cao Z, Lao L, Hou Y, Cui C, Klein WL, Luo Y. Stimulation of neurogenesis and synaptogenesis by bilobalide and quercetin *via* common final pathway in hippocampal neurons. *J. Alzheimers Dis.*, **18**, 787–798 (2009).
- 74) Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H. Multi-function of myricetin on A beta: neuroprotection *via* a conformational change of A beta and reduction of A beta *via* the interference of secretases. *J. Neurosci. Res.*, **86**, 368–377 (2008).
- 75) Ono K, Yoshiike Y, Takashima A, Hasegawa K, Naiki H, Yamada M. Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols *in vitro*: implications for the prevention and therapeutics of Alzheimer's disease. *J. Neurochem.*, **87**, 172–181 (2003).
- 76) Wang QM, Wang GL, Ma ZG. Protective effects of myricetin on chronic stress-induced cognitive deficits. *Neuroreport*, **27**, 652–658 (2016).
- 77) Park SY, Kim HY, Park HJ, Shin HK, Hong KW, Kim CD. Concurrent treatment with taxifolin and cilostazol on the lowering of β -amyloid accumulation and neurotoxicity *via* the suppression of P-JAK2/P-STAT3/NF- κ B/BACE1 signaling pathways. *PLOS ONE*, **11**, e0168286 (2016).
- 78) Sato M, Murakami K, Uno M, Ikubo H, Nakagawa Y, Katayama S, Akagi K, Irie K. Structure–activity relationship for (+)-taxifolin isolated from silymarin as an inhibitor of amyloid β aggregation. *Biosci. Biotechnol. Biochem.*, **77**, 1100–1103 (2013).
- 79) Saito S, Yamamoto Y, Maki T, Hattori Y, Ito H, Mizuno K, Harada-Shiba M, Kalaria RN, Fukushima M, Takahashi R, Ihara M. Taxifolin inhibits amyloid- β oligomer formation and fully restores vascular integrity and memory in cerebral amyloid angiopathy. *Acta Neuropathol Commun.*, **5**, 26 (2017).
- 80) Kuppusamy A, Arumugam M, George S. Combining *in silico* and *in vitro* approaches to evaluate the acetylcholinesterase inhibitory profile of some commercially available flavonoids in the management of Alzheimer's disease. *Int. J. Biol. Macromol.*, **95**, 199–203 (2017).
- 81) Nakagawa T, Itoh M, Ohta K, *et al.* Improvement of memory recall by quercetin in rodent contextual fear conditioning and human early-stage Alzheimer's disease patients. *Neuroreport*, **27**, 671–676 (2016).
- 82) Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*, **425**, 191–196 (2003).
- 83) Donmez G, Wang D, Cohen DE, Guarente LRS. SIRT1 suppresses beta-amyloid production by activating the alpha-secretase gene ADAM10. *Cell*, **142**, 320–332 (2010).
- 84) Julien C, Tremblay C, Emond V, Lebbadi M, Salem N Jr, Bennett DA, Calon F. Sirtuin 1 reduction parallels the accumulation of tau in Alzheimer disease. *J. Neuropathol. Exp. Neurol.*, **68**, 48–58 (2009).
- 85) Choi B, Kim S, Jang BG, Kim MJ. Piceatannol, a natural analogue of resveratrol, effectively reduces β -amyloid levels *via* activation of alpha-secretase and matrix metalloproteinase-9. *J. Funct. Foods*, **23**, 124–134 (2016).
- 86) Chen J, Zhou Y, Mueller-Steiner S, Chen LF, Kwon H, Yi S, Mucke L, Gan L. SIRT1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-kappaB signaling. *J. Biol. Chem.*, **280**, 40364–40374 (2005).
- 87) Feng X, Liang N, Zhu D, Gao Q, Peng L, Dong H, Yue Q, Liu H, Bao L, Zhang J, Hao J, Gao Y, Yu X, Sun J. Resveratrol inhibits β -amyloid-induced neuronal apoptosis through regulation of SIRT1-ROCK1 signaling pathway. *PLOS ONE*, **8**, e59888 (2013).
- 88) Choi CW, Choi YH, Cha MR, Kim YS, Yon GH, Hong KS, Park WK, Kim YH, Ryu SY. *In vitro* BACE-1 inhibitory activity of resveratrol oligomers from the seed extract of *Paeonia lactiflora*. *Planta Med.*, **77**, 374–376 (2011).
- 89) Rivière C, Richard T, Quentin L, Krisa S, Mérillon JM, Monti JP. Inhibitory activity of stilbenes on Alzheimer's beta-amyloid fibrils *in vitro*. *Bioorg. Med. Chem.*, **15**, 1160–1167 (2007).
- 90) Vingetdeux V, Giliberto L, Zhao H, Chandakkar P, Wu Q, Simon JE, Janle EM, Lobo J, Ferruzzi MG, Davies P, Marambaud P. AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism. *J. Biol. Chem.*, **285**, 9100–9113 (2010).
- 91) Marambaud P, Zhao H, Davies P. Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. *J. Biol. Chem.*, **280**, 37377–37382 (2005).
- 92) Varamini B, Sikilidis AK, Bradford KL. Resveratrol increases cerebral glycogen synthase kinase phosphorylation as well as protein levels of drebrin and transthyretin in mice: an exploratory study. *Int. J. Food Sci. Nutr.*, **65**, 89–96 (2014).
- 93) Virgili M, Contestabile A. Partial neuroprotection of *in vivo* excitotoxic brain damage by chronic administration of the red wine antioxidant agent, *trans*-resveratrol in rats. *Neurosci. Lett.*, **281**, 123–126 (2000).
- 94) Feng Y, Wang XP, Yang SG, Wang YJ, Zhang X, Du XT, Sun XX, Zhao M, Huang L, Liu RT. Resveratrol inhibits beta-amyloid oligomeric cytotoxicity but does not prevent oligomer formation. *Neurotoxicology*, **30**, 986–995 (2009).
- 95) Jang MH, Piao XL, Kim JM, Kwon SW, Park JH. Inhibition of cholinesterase and amyloid-beta aggregation by resveratrol oligomers from *Vitis amurensis*. *Phytother. Res.*, **22**, 544–549 (2008).
- 96) Tsai RY, Chou KY, Shen CH, Chien CC, Tsai WY, Huang YN, Tao PL, Lin YS, Wong CS. Resveratrol regulates N-methyl-D-aspartate receptor expression and suppresses neuroinflammation in morphine-tolerant rats. *Anesth. Analg.*, **115**, 944–952 (2012).
- 97) Wang G, Chen L, Pan X, Chen J, Wang L, Wang W, Cheng R, Wu F, Feng X, Yu Y, Zhang HT, O'Donnell JM, Xu Y. The effect of resveratrol on beta amyloid-induced memory impairment involves

- inhibition of phosphodiesterase-4 related signaling. *Oncotarget*, **7**, 17380–17392 (2016).
- 98) Turner RS, Thomas RG, Craft S, van Dyck CH, Mintzer J, Reynolds BA, Brewer JB, Rissman RA, Raman R, Aisen PS. A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease. *Neurology*, **85**, 1383–1391 (2015).
 - 99) de Wilde MC, Vellas B, Girault E, Yavuz AC, Sijben JW. Lower brain and blood nutrient status in Alzheimer's disease: Results from meta-analyses. *Alzheimer's Dement. (N Y)*, **3**, 416–431 (2017).
 - 100) Grimm MO, Kuchenbecker J, Grösgen S, Burg VK, Hundsdoerfer B, Rothhaar TL, Friess P, de Wilde MC, Broersen LM, Penke B, Péter M, Vigh L, Grimm HS, Hartmann T. Docosahexaenoic acid reduces amyloid beta production via multiple pleiotropic mechanisms. *J. Biol. Chem.*, **286**, 14028–14039 (2011).
 - 101) Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L, LaFerla FM. Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-beta and tau pathology via a mechanism involving presenilin 1 levels. *J. Neurosci.*, **27**, 4385–4395 (2007).
 - 102) Hashimoto M, Shahdat HM, Yamashita S, Katakura M, Tanabe Y, Fujiwara H, Gamoh S, Miyazawa T, Arai H, Shimada T, Shido O. Docosahexaenoic acid disrupts *in vitro* amyloid beta(1-40) fibrillation and concomitantly inhibits amyloid levels in cerebral cortex of Alzheimer's disease model rats. *J. Neurochem.*, **107**, 1634–1646 (2008).
 - 103) Lee AY, Lee MH, Lee S, Cho EJ. Alpha-linolenic acid from *Perrilla frutescens* var. *japonica* oil protects A β -induced cognitive impairment through regulation of APP processing and A β degradation. *J. Agric. Food Chem.*, **65**, 10719–10729 (2017).
 - 104) Hooijmans CR, Rutters F, Dederen PJ, Gambarota G, Veltien A, van Groen T, Broersen LM, Lütjohann D, Heerschap A, Tanila H, Kiliaan AJ. Changes in cerebral blood volume and amyloid pathology in aged Alzheimer APP/PS1 mice on a docosahexaenoic acid (DHA) diet or cholesterol enriched Typical Western Diet (TWD). *Neurobiol. Dis.*, **28**, 16–29 (2007).
 - 105) Bazan NG. Neuroprotectin DI (NPD1): a DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress. *Brain Pathol.*, **15**, 159–166 (2005).
 - 106) Florent S, Malaplate-Armand C, Youssef I, Kriem B, Koziel V, Escanyé MC, Fife A, Sponne I, Leininger-Muller B, Olivier JL, Pillot T, Oster T. Docosahexaenoic acid prevents neuronal apoptosis induced by soluble amyloid-beta oligomers. *J. Neurochem.*, **96**, 385–395 (2006).
 - 107) Hashimoto M, Hossain S, Shimada T, Shido O. Docosahexaenoic acid-induced protective effect against impaired learning in amyloid beta-infused rats is associated with increased synaptosomal membrane fluidity. *Clin. Exp. Pharmacol. Physiol.*, **33**, 934–939 (2006).
 - 108) Minami M, Kimura S, Endo T, Hamaue N, Hirafuji M, Togashi H, Matsumoto M, Yoshioka M, Saito H, Watanabe S, Kobayashi T, Okuyama H. Dietary docosahexaenoic acid increases cerebral acetylcholine levels and improves passive avoidance performance in stroke-prone spontaneously hypertensive rats. *Pharmacol. Biochem. Behav.*, **58**, 1123–1129 (1997).
 - 109) Calon F, Lim GP, Morigahara T, Yang F, Ubéda O, Salem N Jr, Frautschy SA, Cole GM. Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. *Eur. J. Neurosci.*, **22**, 617–626 (2005).
 - 110) Choi JE, Park Y. EPA and DHA, but not ALA, have antidepressant effects with 17 β -estradiol injection via regulation of a neurobiological system in ovariectomized rats. *J. Nutr. Biochem.*, **49**, 101–109 (2017).
 - 111) Song C, Shieh CH, Wu YS, Kalueff A, Gaikwad S, Su KP. The role of omega-3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids in the treatment of major depression and Alzheimer's disease: Acting separately or synergistically? *Prog. Lipid Res.*, **62**, 41–54 (2016).
 - 112) Li YC, Chen Q, Wan XZ, Yang XL, Liu X, Zhong L. Effects of conjugated linoleic acid on cleavage of amyloid precursor protein via PPAR γ . *Neurol. Sci.*, **32**, 1095–1101 (2011).
 - 113) Youn K, Yun EY, Lee J, Kim JY, Hwang JS, Jeong WS, Jun M. Oleic acid and linoleic acid from *Tenebrio molitor* larvae inhibit BACE1 activity *in vitro*: molecular docking studies. *J. Med. Food*, **17**, 284–289 (2014).
 - 114) Liu Y, Yang L, Conde-Knappe K, Behr D, Shearman MS, Shachter NS. Fatty acids increase presenilin-1 levels and [gamma]-secretase activity in PSwt-1 cells. *J. Lipid Res.*, **45**, 2368–2376 (2004).
 - 115) Tang KS. Protective effect of arachidonic acid and linoleic acid on 1-methyl-4-phenylpyridinium-induced toxicity in PC12 cells. *Lipids Health Dis.*, **13**, 197 (2014).
 - 116) Joo NE, Park CS. Inhibition of excitotoxicity in cultured rat cortical neurons by a mixture of conjugated linoleic acid isomers. *Pharmacol. Res.*, **47**, 305–310 (2003).
 - 117) Nishizaki T, Ikeuchi Y, Matsuoka T, Sumikawa K. Short-term depression and long-term enhancement of ACh-gated channel currents induced by linoleic and linolenic acid. *Brain Res.*, **751**, 253–258 (1997).
 - 118) Lee AY, Choi JM, Lee J, Lee MH, Lee S, Cho EJ. Effects of vegetable oils with different fatty acid compositions on cognition and memory ability in A β 25–35-induced Alzheimer's disease mouse model. *J. Med. Food*, **19**, 912–921 (2016).
 - 119) García-Hernández VM, Gallar M, Sánchez-Soriano J, Micol V, Roche E, García-García E. Effect of omega-3 dietary supplements with different oxidation levels in the lipidic profile of women: a randomized controlled trial. *Int. J. Food Sci. Nutr.*, **64**, 993–1000 (2013).
 - 120) Ottestad I, Vogt G, Retterstøl K, Myhrstad MC, Haugen JE, Nilsson A, Ravn-Haren G, Nordvi B, Brønner KW, Andersen LF, Holven KB, Ulven SM. Oxidised fish oil does not influence established markers of oxidative stress in healthy human subjects: a randomised controlled trial. *Br. J. Nutr.*, **108**, 315–326 (2012).
 - 121) Staprans I, Rapp JH, Pan XM, Kim KY, Feingold KR. Oxidized lipids in the diet are a source of oxidized lipid in chylomicrons of human serum. *Arterioscler. Thromb.*, **14**, 1900–1905 (1994).
 - 122) Moré MI, Freitas U, Rutenberg D. Positive effects of soy lecithin-derived phosphatidylserine plus phosphatidic acid on memory, cognition, daily functioning, and mood in elderly patients with Alzheimer's disease and dementia. *Adv. Ther.*, **31**, 1247–1262 (2014).
 - 123) Growdon JH. Use of phosphatidylcholine in brain diseases: an overview. *Lecithin: technological, biological, and therapeutic aspects*. Plenum Press, New York, pp.121–136 (1987).
 - 124) Blusztajn JK, Liscovitch M, Mauron C, Richardson UI, Wurtman RJ. Phosphatidylcholine as a precursor of choline for acetylcholine synthesis. *J. Neural Transm. Suppl.*, **24**, 247–259 (1987).
 - 125) Ko M, Hattori T, Abdullah M, Gong JS, Yamane T, Michikawa M. Phosphatidylcholine protects neurons from toxic effects of amyloid β -protein in culture. *Brain Res.*, **1642**, 376–383 (2016).
 - 126) Higgins JP, Flicker L. Lecithin for dementia and cognitive impairment. *Cochrane Database Syst. Rev.*, **3**, CD001015 (2003).
 - 127) Farooqui AA, Rapoport SI, Horrocks LA. Membrane phospholipid alterations in Alzheimer's disease: deficiency of ethanolamine plasmalogens. *Neurochem. Res.*, **22**, 523–527 (1997).
 - 128) Wood PL, Mankidy R, Ritchie S, Heath D, Wood JA, Flax J, Goodenowe DB. Circulating plasmalogen levels and Alzheimer disease assessment scale—cognitive scores in Alzheimer patients. *J. Psychiatry Neurosci.*, **35**, 59–62 (2010).
 - 129) Fujino T, Yamada T, Asada T, Tsuboi Y, Wakana C, Mawatari S, Kono S. Efficacy and blood plasmalogen changes by oral administration of plasmalogen in patients with mild Alzheimer's disease and mild cognitive impairment: a multicenter, randomized, double-blind, placebo-controlled trial. *EBioMedicine*, **17**, 199–205 (2017).
 - 130) Rothhaar TL, Grösgen S, Haupenthal VJ, Burg VK, Hundsdoerfer

- B, Mett J, Riemenschneider M, Grimm HS, Hartmann T, Grimm MO. Plasmalogens inhibit APP processing by directly affecting γ -secretase activity in Alzheimer's disease. *ScientificWorldJournal*, **2012**, 141240 (2012).
- 131) Katafuchi T, Ifuku M, Mawatari S, Noda M, Miake K, Sugiyama M, Fujino T. Effects of plasmalogens on systemic lipopolysaccharide-induced glial activation and β -amyloid accumulation in adult mice. *Ann. N. Y. Acad. Sci.*, **1262**, 85–92 (2012).
- 132) Périchon R, Moser AB, Wallace WC, Cunningham SC, Roth GS, Moser HW. Peroxisomal disease cell lines with cellular plasmalogen deficiency have impaired muscarinic cholinergic signal transduction activity and amyloid precursor protein secretion. *Biochem. Biophys. Res. Commun.*, **248**, 57–61 (1998).
- 133) Palizgir MT, Akhtari M, Mahmoudi M, Mostafaei S, Rezaeiemanesh A, Shahram F. Curcumin reduces the expression of interleukin 1β and the production of interleukin 6 and tumor necrosis factor alpha by M1 macrophages from patients with Behcet's disease. *Immunopharmacol. Immunotoxicol.*, **40**, 297–302 (2018).
- 134) Mori T, Koyama N, Tan J, Segawa T, Maeda M, Town T. Combined treatment with the phenolics (–)-epigallocatechin-3-gallate and ferulic acid improves cognition and reduces Alzheimer-like pathology in mice. *J. Biol. Chem.*, **294**, 2714–2731 (2019).
- 135) Mori T, Koyama N, Tan J, Segawa T, Maeda M, Town T. Combination therapy with octyl gallate and ferulic acid improves cognition and neurodegeneration in a transgenic mouse model of Alzheimer's disease. *J. Biol. Chem.*, **292**, 11310–11325 (2017).