Protective Effects of Ferulic Acid in Amyloid Precursor Protein Plus Presenilin-1 Transgenic Mouse Model of Alzheimer Disease

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We previously reported the protective effects of long-term administration of ferulic acid against the in vivo toxicity of β-amyloid peptide administered intracerebroventricularly in mice. In the present study, we investigated the effects of ferulic acid in transgenic amyloid precursor protein (APP)swe/presenilin 1 (PS1)dE9 (APP/PS1) mouse model of Alzheimer disease (AD). Chronic (for 6 months from the age of 6 to 12 months) oral administration of ferulic acid at a dose of 5.3 mg/kg/day significantly enhanced the performance in novel-object recognition task, and reduced amyloid deposition and interleukin-1 beta (IL-1β) levels in the frontal cortex. These results suggest that ferulic acid at a certain dosage could be useful for prevention and treatment of AD.

Key words β-amyloid peptide; Alzheimer disease; amyloid precursor protein; presenilin 1; ferulic acid

Abnormal accumulation of β-amyloid peptide (Aβ) in the brain is regarded to be important in the pathogenesis of Alzheimer disease (AD), a progressive neurodegenerative disorder.1,2) Thus, continuing efforts have been made to develop strategies targeting Aβ for prevention and treatment of AD.3)

Ferulic acid is a natural molecule abundantly present in the various plants. It has anti-oxidant4,5) and anti-inflammatory6,7) activities. We previously reported that long-term (4 weeks) administration of ferulic acid protects against intracerebroventricularly (i.c.v.) administered Aβ1–42-induced learning and memory impairment5) and astrocyte and microglial activation8–10) in mice. Furthermore, ferulic acid ethyl ester was reported to protect against Aβ1–42-induced oxidative stress both in vitro11) and in vivo.12) Ferulic acid was also reported to destabilize preformed Aβ fibrils in vitro.13)

In addition to the i.c.v. injection of Aβ1–42,8) amyloid precursor protein (APP) transgenic4) or APP/presenilin 1 (PS1) double transgenic15) mice have been very useful for evaluation of beneficial effects of substances targeting Aβ in vivo. Thus, in the present study, we wanted to examine the effects of ferulic acid on the APP/PS1 double transgenic mice, extending our previously study on the protective effects of ferulic acid against the i.c.v. administered Aβ toxicity.8–10)

MATERIALS AND METHODS

Mice and Ferulic Acid Female APP/PS1 (APPswe/PS1dE9) transgenic mice were obtained from Jackson Laboratory (Bar Harbor, ME, U.S.A.). Procedures for animal experiments were approved by the Animal Experimentation Committee at Hallym University. Ferulic acid was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Because 0.004% and 0.006% of ferulic acid in the drinking water (with the average water intake per mouse per day of about 6–8 mL) found to be effective in our previous study on the protective effects of long-term administration of ferulic acid against the in vivo toxicity of β-amyloid peptide administered intracerebroventricularly in mice8–10) was approximately 9–13 mg/kg/d (for 0.004%) and 14–19 mg/kg/d (for 0.006%), we chose the ferulic acid doses of 5.3 and 16 mg/kg/d in the present study. Ferulic acid was administered from 6 months of age for 6 months at 2 different doses (5.3 or 16 mg/kg/d) (Fig. 1). Each dose of ferulic acid was added to the chow as calculated for each mouse to take the dose. All the five mice per group at the start survived the 6-month treatment. At 6 months after the start of ferulic acid treatment, i.e. at 12 months of age, mice were put to behavioral tests, and then were killed to obtain frontal cortex to assay Aβ (Aβ1→42 and Aβ1→40) levels and interleukin-1 beta (IL-1β) levels.

![Fig. 1. Experimental Schedules for Evaluation of the Effects of Ferulic Acid on the Mouse Models of APP/PS1-Transgenic Mice](image-url)

Ferulic acid was administered from 6 months of age for 6 months at 2 different doses (5.3 or 16 mg/kg/d). Behavioral tests and measurement of Aβ and IL-1β were done on the indicated time points.

The authors declare no conflict of interest.

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Behavioral Tests  Y-maze task was done as described previously. Novel-object recognition task was executed as previously described. Briefly, mice were exposed to empty cage for 5 min for 2 consecutive days to accommodate to the experiment cage. On the third and fourth day, mice were exposed to a cage with 2 objects. On the fifth day, one of the objects was replaced with a new one.

Measurement of Aβ and IL-1β  Cortex (15–20 mg) was homogenized at 100 mg cortex/mL Tris buffer (25 mM, pH 7.6) (NaCl 8 g/L, KCl 0.2 g/L, Trisma base 3 g/L) supplemented with ethylenediaminetetraacetic acid (EDTA) 1 mM, protease inhibitor cocktail (1 mL/100 mL), phenylmethylsulfonyl fluoride (PMSF) 0.5 mM, and leupetin 1 mg/mL. Homogenate was centrifuged at 10000×g for 20 min, and supernatant was used for cytokine measurement. Pellet was re-suspended in Tris buffer (25 mM) supplemented with guanidine HCl 5 M, and protease inhibitors cocktail (1 mL/100 mL), and after sonication, was centrifuged at 10000×g for 20 min, and supernatant was used for Aβ measurement. Dilution buffer was Tris buffer containing 5% bovine serum albumin (BSA) and 0.03% Tween 20. Aβ1−42, Aβ1−40, and IL-1β were assayed with immunossay kits (Biosource). Assays were performed according to the manufacturer’s instructions.

Statistics  Statistical analysis was carried out by one-way analysis of variance (ANOVA). Bonferroni test was used for post-hoc comparisons. p values less than 0.05 were considered to indicate statistical significance.

RESULTS  Chronic administration of ferulic acid for 6 months tended to increase body weight (29.0±2.0, 35.2±2.7, 37.0±3.0 g for control, 5.3 mg/kg/d ferulic acid, 16 mg/kg/d ferulic acid, respectively, n=5) but the increase did not reach statistical significance (ANOVA). APP/PS1-Transgenic mice at the age of 12 months had no significant changes in spontaneous alternation behavior in Y-maze test (data not shown). In the novel-object recognition task, treatment with ferulic acid at a dose of 5.3 mg/kg/d for 6 months significantly enhanced the performance in the APP/PS1-transgenic mice (Fig. 2). However, ferulic acid at a dose of 16 mg/kg/day was ineffective.

Next, we examined the effect of ferulic acid treatment for 6 months on the Aβ1−40 and Aβ1−42 levels in APP/PS1-transgenic mice at 12 months of age. As shown in Fig. 3A, treatment with ferulic acid induced a significant decrease in cortical Aβ1−40 levels; ferulic acid was more effective at the lower dose (5.3 mg/kg/d). For hippocampus, a similar inhibitory pattern appeared, but the inhibition did not reach statistical significance. For Aβ1−42 levels, the inhibition by ferulic acid was less prominent, and only the lower dose (5.3 mg/kg/d) of ferulic acid was effective in the frontal cortex (Fig. 3B).

Next, we examined the effect of ferulic acid treatment on cortical IL-1β levels. As shown in Fig. 4, treatment with ferulic acid induced a significant decrease in cortical IL-1β levels at both the lower and the higher doses. However, it is to be noted that in both the behavioral (Fig. 2) and the biochemical (Figs. 3, 4) analyses, the lower dose (5.3 mg/kg/day) of ferulic acid was more effective.

DISCUSSION  In the present study, we found that chronic administration of ferulic acid for 6 months improved novel-object recognition test (Fig. 2), and reduced cortical Aβ1−40 and Aβ1−42 levels (Fig. 3) and decreased IL-1β levels (Fig. 4) in APP/PS1 mice. The close ferulic acid dose–response relationships between novel object recognition test (Fig. 2) and Aβ deposition/IL-1β levels (Figs. 3, 4) strongly suggests that the behavioral improvement results from lowered Aβ/IL-1β levels.

Previously we reported that ferulic acid administration protects against i.c.v. administered Aβ toxicities in vivo. Ferulic acid inhibits Aβ deposition in the cortex. Thus, it is suggested that ferulic acid has multiple modes of action regarding Aβ in the AD pathogenesis. Although the mechanism of the ferulic acid-induced inhibition of Aβ deposition in the cortex was not addressed in the present study, it could be due to ferulic acid’s anti-oxidant and anti-inflammatory activities. However, the reason why ferulic acid decreases Aβ deposition more effectively in the frontal cortex than the hippocampus (Fig. 3) needs to be clarified.

Of the two doses of ferulic acid used in this study, ferulic acid’s beneficial effects was observed only at the lower dose (5.3 mg/kg/d) (Fig. 2), while for Aβ1−40 deposition/IL-1β levels in the frontal cortex the higher dose (16 mg/kg/d) also showed a significant but less prominent effect (Figs. 3A, 4). These results suggest that the beneficial effects of ferulic acid can be observed only at a certain dosage range. Recently, it has been reported that chronic administration of ferulic acid did not affect Aβ deposition in the brain of APP/PS1 mice. These mice were fed with chow containing 0.5% ferulic acid for 1 year (from the age of 4 months to 14 months). If each mouse is assumed to take about 3–4 g of Chow (containing 0.5% ferulic acid) per day, the ferulic acid dose is around 500–667 mg/kg/d, which about 95–125 fold higher than the dose of 5.3 mg/kg/d used in the present study. Thus, the
reason for the lack of ferulic acid effect on Aβ deposition in Hamaguchi et al. most probably could be due to the use of an inappropriately high dose. It is to be noted that curcumin, an analogue of ferulic acid, has also been reported to decrease amyloid deposition and IL-1β levels in the brain of APP transgenic mice at a low dose but not at a high dose. The limitation of the present study is that we used only female APP/PS1 Tg mice. The effect of ferulic acid needs to be also studied in the male APP/PS1 Tg mice to examine a possible gender-specific effect of ferulic acid.

In conclusion, administration of ferulic acid showed protective activities in APP/PS1 transgenic mice, including the decrease in amyloid deposition in the brain (Figs. 2–4). These results suggest that ferulic acid could be useful for prevention and treatment of AD, warranting a clinical study in the AD patients.

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