Inhibitory Effects of Long-Term Administration of Ferulic Acid on Microglial Activation Induced by Intracerebroventricular Injection of β-Amyloid Peptide (1—42) in Mice

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Flavonoids and monophenolic compounds have been well described in recent years as antioxidants and scavengers of reactive oxygen and nitrogen species. In the present study, we aimed to characterize the effects of long-term administration of ferulic acid on the centrally administered β-amyloid peptide (Aβ1—42)-induced activation of microglial cells in mice. Aβ1—42 increased the immunoreactivity of OX-42, a microglial marker, and interferon-γ in the hippocampus at 8 h after the intracerebroventricular injection. The effects were suppressed by long-term (4-week) pretreatment with ferulic acid. This inhibition of microglial cell activation may underlie the beneficial effects of long-term administration of ferulic acid on Aβ1—42-induced toxicity in vivo.

Key words β-amyloid peptide (Aβ); microglia; endothelial nitric oxide synthase; ferulic acid; Alzheimer’s disease

Materials and Methods

Materials Male ICR mice weighing 18—26 g at the beginning of experiments (Myung-Jin, Inc., Seoul, Korea) were used in all experiments. The mice were housed 5 per cage in a room maintained at 22±1°C with an alternating 12-h light–dark cycle. Food was available ad libitum. Aβ1—42 (American Peptide Company, U.S.A.) and Aβ42–41 (Bachem, Switzerland) were prepared as stock solutions at a concentration of 37 μg/μl in sterile 0.1 M phosphate-buffered saline (PBS) (pH 7.4) and aliquots were stored at −20°C until use. Ferulic acid (Sigma) was dissolved each day in tap water at a concentration of 0.006% (w/v). Assay with HPLC with electrochemical detection showed 11% loss of ferulic acid (0.006% w/v) in tap water in 24 h at room temperature.

Experimental Design Mice were allowed free access to normal drinking water or ferulic acid solution for 4 weeks. Subsequently, Aβ1—42 was administered via intracerebroventricular injection, and at various time points after the Aβ1—42 injection the brains were analyzed immunohistochemically.

Control animals were injected with Aβ42–41.

Intracerebroventricular Injection of Aβ1—42 The administration of Aβ1—42 was performed according to the procedure established by Laursen and Belknap. 5 Briefly, each mouse was injected at the bregma with a 50-μl Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.4 mm. The injection volume was 5 μl.

Immunocytochemistry At various time points after injection of Aβ1—42, mice were transcardially perfused and postfixed for 4 h in 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose, sectioned coronally (45 μm) on a freezing microtome, and collected in a cryoprotectant for storage at −20°C. Floating sections of brains were processed as described previously. 5 After 3 10-min rinses in PBS, sections were placed in the cryoprotectant, preincubated for 30 min in 0.1 M PBS with 1% bovine serum albumin and 0.2% Triton X-100, and incubated overnight with the following primary antisera: anti-rat interferon (IFN)-γ (1:500, R&D); and anti-rat OX-42 (1:500, Harlan Sera-Labs). On the following day, the sections were incubated for 1 h in biotinylated rabbit and goat secondary antibody obtained from Vector Laboratories. After incubation with the Vector Elite ABC kit, antigens were detected with 3,3-diaminobenzidine tetrahydrochloride (DAB) as the chromogen.

RESULTS

Inhibition of Aβ1—42-Induced Increase in OX-42 Immunoreactivity by Ferulic Acid To examine whether central Aβ1—42 injection induces microglial activation, OX-42 immunoreactivity, a marker of activated microglia, was examined. OX-42 immunoreactivity was markedly enhanced 8 h after injection of Aβ1—42 (Fig. 1b), and returned to basal levels at 1 d (data not shown). In animals pretreated with ferulic acid for 4 weeks, the Aβ1—42-induced increases in hippocampal OX-42 immunoreactivity were effectively blocked (Fig. 1c).

Inhibition of Aβ1—42-Induced Increase in IFN-γ Immunoreactivity by Ferulic Acid Interestingly, the time
immunoreactivity is increased at 8 h after central injection of central A
GFAP and eNOS immunoreactivities peaked 3 or 5 d after the same as OX-42 immunoreactivity. IFN-
g–immunoreactivity in the hippocampus was examined 8 h after injection of 410 pmol of A
m.
1—42 (B, C, E, F). Mice injected with A
m.
1—42 410 pmol served as controls (A, B). A, B, and C are OX-42 immunoreactivity in the hippocampus in control, A
m.
1—42 and FA/A
m.
1—42 repectively. D, E, and F are IFN-
g–immunoreactivity in the hippocampus in control, A
m.
1—42 and FA/A
m.

The time course of IFN-γ immunoreactivity in the hippocampus was the same as OX-42 immunoreactivity. IFN-γ-immunoreactive cells appeared to be microglial cells. In animals pre-
treated with ferulic acid for 4 weeks, the Aβ1–42–induced increases in hippocampal IFN-γ immunoreactivity were effective-
ly blocked (Fig. 1f).

DISCUSSION

Previously, we reported that a single intracerebroventricular injection of a picomolar dose (410 pmol) of Aβ1–42 in mice effectively activates astrocytes, as evidenced by increased immunoreactivities of GFAP and IL-1β, which were blocked by long-term pretreatment with ferulic acid.29 In the present study, we extended our previous findings by showing that centrally injected Aβ1–42 induces activation of microglia, as evidenced by the increased immunoreactivity of OX-42, which was also effectively inhibited by ferulic acid.

We found that activation of microglia is more rapid com-
pared with that of astrocytes, i.e., we observed that OX-42 immunoreactivity is increased at 8 h after central injection of Aβ1–42, which returned to the basal state at 24 h, while GFAP and eNOS immunoreactivities peaked 3 or 5 d after central Aβ1–42 injection.5 Thus it appears likely that the mi-
croglia is activated at 8 h after Aβ1–42 injection, which returns to the basal state 24 h after injection, while astrocyte activation is slower, which peaks post-Aβ1–42 injection day 3 to 5. In addition, activated microglia exhibits increased IFN-γ immunoreactivity. The time course of IFN-γ immunostaining is the same as that of OX-42. IFN-γ could act as an inflam-
matory amplifier aggravating the neurodegenerative process by priming microglia or monocytes/macrophages for the se-
cretion of proinflammatory cytokines.7 Further, IFN-γ was reported to show a synergistic effect on microglia activation in response to Aβ.9 Although the mechanism of astrocyte ac-
tivation is unclear at present, it can be speculated that mi-
croglia activation induces subsequent astrocyte activation, because the time course of astrocyte activation follows mi-
croglia activation. The primary site of action of ferulic acid could be microglia. Thus it may be suggested that long-term administration of ferulic acid could be microglia. Thus it may be suggested that long-term administration of ferulic acid induces microglia to suppress the Aβ1–42 stimulus.

Activation of glial cells including increased eNOS expression by preconditioning stimulus was postulated to be re-
ponsible for the induction of tolerance to neuronal damage.5,9,10 In our laboratory, we noted a transient and slight increase in IL-1β and eNOS (unpublished data) immunoreac-
tivity in astrocytes on day 14 after treatment with ferulic acid, which returned to basal levels on day 28. Thus it can be speculated that activation and subsequent recovery of glial cells by ferulic acid might somehow induce resistance to the subsequent Aβ1–42 insult to the brain. Further study is needed to explore the exact mechanisms of ferulic acid in neuroprotection against Aβ1–42.

In conclusion, long-term administration of ferulic acid prevented the Aβ1–42–induced activation of microglia. This inhibition of microglial cell activation may underlie the benefi-
cial effect of long-term administration of ferulic acid against Aβ1–42–induced toxicity in vivo.

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REFERENCES