Effects of Flavonoid Compounds on β-amyloid-peptide-induced Neuronal Death in Cultured Mouse Cortical Neurons

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Excessive accumulation of β-amyloid peptide (Aβ) is one of the major mechanisms responsible for neuronal death in Alzheimer's disease. Flavonoids, primarily antioxidants, are a group of polyphenolic compounds synthesized in plant cells. The present study aimed to identify flavonoid compounds that could inhibit Aβ-induced neuronal death by examining the effects of various flavonoids on the neurotoxicity of Aβ fragment 25-35 ($A\beta_{25-35}$) in mouse cortical cultures. $A\beta_{25-35}$ induced concentration- and exposure-time-dependent neuronal death. Neuronal death induced by 20 μM $A\beta_{25-35}$ was significantly inhibited by treatment with either Trolox or ascorbic acid. Among 10 flavonoid compounds tested [apigenin, baicalein, catechin, epicatechin, epigallocatechin gallate (EGCG), kaempferol, luteolin, myricetin, quercetin, and rutin], all except apigenin showed strong 1,1-diphenyl-2-pycrylhydrazyl (DPPH) scavenging activity under cell-free conditions. The flavonoid compounds except apigenin at a concentration of 30 μM also significantly inhibited neuronal death induced by 20 μM $A\beta_{25-35}$ at the end of 24 hours of exposure. Epicatechin, EGCG, luteolin, and myricetin showed more potent and persistent neuroprotective action than did the other compounds. These results demonstrated that oxidative stress was involved in Aβ-induced neuronal death, and antioxidative flavonoid compounds, especially epicatechin, EGCG, luteolin, and myricetin, could inhibit neuronal death. These findings suggest that these four compounds may be developed as neuroprotective agents against Alzheimer's disease.

Key Words: *β-Amyloid peptide; Alzheimer's disease; Flavonoids*

INTRODUCTION

Flavonoids make up the most common group of polyphenolic compounds in the human diet that are synthesized in plant cells. Flavonoids have a 15-carbon skeleton consisting of two fused six-membered rings (an aromatic ring and a heterocyclic ring) connected through a carbon-carbon bridge to an aromatic ring.[1] The flavonoids can be divided into six different major classes (flavonols, flavanones, flavones, isoflavones, catechins, and anthocyanidins) on the basis of differences in the structure of their molecular backbone.[2,3] The major sources of flavonoids include fruits, vegetables, tea, wine, and cocoa.[4] The flavonol quercetin and the flavone apigenin are found in onions, apples, broccoli, and berries. The flavanone naringenin is found in citrus foods. Catechins are abundant in green tea. Cyanidin and other anthocyanidins are largely responsible for the deep colors of berries, grapes, and red wine. Genistein is an isoflavone found predominantly in legumes. The flavonoid consumed most, in general, is quercetin, and the richest sources of flavonoids consumed in general are tea, onions, and apples.[5] Flavonoids contain a number of phenolic hydroxyl groups attached to ring structures, which may act as antioxidants,[6-8] as radical-scavenging agents,[9,10] or as agents of anti-inflammation.[11,12] Recently, significant evidence has emerged to indicate that the consumption of flavonoid-rich foods is associated with lower rates of dementia[13] and beneficial effects on memory and learning.[13,14]

Alzheimer's disease (AD) is the major subtype of de-

mentia in all age groups.[15] AD is a progressive neuro-degenerative disorder associated with a disruption of neuronal function and a clinically gradual deterioration in remembering recent events, thinking abilities, and behavior. AD is pathologically characterized by the presence of neuritic plaques, neurofibrillary tangles, and the loss of cortical neurons and synapses,[16] which is a hallmark feature of the AD brain. The β-amyloid peptide (Aβ) is the major component of extracellular neuritic plaques. Aβ is 39 to 43 amino acid fragments in length and is generated by proteolytic cleavage of the larger amyloid precursor protein (APP), a ubiquitously expressed transmembrane glycoprotein.[17,18] An excessive accumulation of Aβ is one of the significant factors responsible for neuronal death in AD.[19,20]

Among the Aβ fragments (Aβ$_{25\text{-}35}$, Aβ$_{1\text{-}40}$, and Aβ$_{1\text{-}42}$) that have toxic effects on neurons in vitro,[21-24] Aβ$_{25\text{-}35}$ is one of the short peptide sequences that retains biological activity comparable with that of full-length Aβ.[25] Although the precise mechanisms mediating Aβ-induced neuronal death have yet to be fully established, it is well known that Aβ-induced neuronal death is associated with the generation of reactive oxygen species in the brain.[26] Also, Aβ initiates a cascade of intracellular events that culminates in neuronal death via calcium-permeable cation channels as well as free-radical-like action.[23,24,27,28]

To determine the antioxidant potencies of 10 types of flavonoids in vitro, and to identify some flavonoid compounds that could inhibit Aβ-induced neuronal death, we investigated the effects of various flavonoids on neuronal death induced by Aβ$_{25\text{-}35}$ in mouse cortical cultures.

MATERIALS AND METHODS

1. Materials

Eagle's minimal essential medium (MEM) and Hanks' balanced solution were purchased from Gibco (Gaithersburg, MD, USA). Fetal bovine serum (FBS) and horse serum were obtained from Hyclone (Logan, UT, USA). HEPES (acid), glucose, NaHCO$_3$, NaCl, KCl, MgCl$_2$, CaCl$_2$, NaOH, phenol red, trypsin, cytosine arabinoside, epidermal growth factor, sucrose, ascorbic acid, cycloheximide, apigenin, baicalein, catechin, epicatechin, epigallocatechin gallate (EGCG), kaempferol, luteolin, myricetin, quercetin, rutin, nifedipine, flunarizine, diltiazem, NMDA, MK-801, Trolox, and Aβ$_{25\text{-}35}$ were all purchased from Sigma-Aldrich (St. Louis, MO, USA).

2. Methods

1) Cortical cell cultures: Mixed cortical cell cultures, including both neurons and glia, were prepared with minor modification of the methods described by Choi et al.[29] Pregnant mice (Institute of Cancer Research, London, UK) at 15 to 17 days of gestation were euthanized by cervical dislocation under halothane anesthesia. Fetal mice were rapidly removed and decapitated. Mouse brains were excised and then rinsed in cold Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution supplemented with 5 mg/mL glucose,

7 mg/mL sucrose, and 0.35 mg/mL sodium bicarbonate (DM). By use of fine-tipped forceps and a microsurgical knife, the meninges were carefully removed from the brain tissue under a stereomicroscope. The cerebral cortex was dissected free and minced into 1-2 mm^3 sized pieces with a sterile scalpel. The cortex pieces were incubated in DM with 0.25% trypsin added at 37°C for 15 minutes and then centrifuged at 1000×g for 5 minutes. After removal of supernatant, the tissue pellet was suspended in 1-2 mL plating medium with Eagle's MEM (Gibco, Gaithersburg, MD) containing 2 mM glutamine, 5% FBS, and 5% horse serum. Cells were separated by 8 or 10 trituration passages by using a flame-narrowed pipette. Dissociated cortical cells were plated onto the previously established glial layer in 24-well multi-well plates at a density of 3 hemispheres/plate (approximately 2.5×10^5 cells per well). The plates were plated in an incubator (Forma Company, USA) at 37°C, 5% CO$_2$, with humidified air. Cytosine arabinoside was added to produce a final concentration of 10 μM at 5 days in vitro and the plates were maintained for 2 days to halt non-neuronal cell division. The culture medium was changed twice a week after 7 days in vitro. Cultures were used at 13 or 14 days in vitro for the experiments.

Cortical glial cultures were prepared from postnatal mice (Institute of Cancer Research, London, UK) aged 1 to 2 days. Dissection and dissociation were as described above for mixed cortical cell cultures, and cells were plated in 24-well multi-well plates at a density of 0.5 hemisphere/plate. The plating medium was supplemented with Eagle's MEM containing 2 mM glutamine, 10% FBS, 10% horse serum, and 10 ng/mL of epidermal growth factor. The plates were maintained in the same incubator. The culture medium was changed once a week after 14 days in vitro. Glial cultures were used for the plating of mixed cortical cell cultures between 18 and 24 days in vitro.

The procedures involving experimental animals complied with the regulations for the care and use of laboratory animals of the animal ethical committee of Chonnam National University.

2) Neurotoxicity and flavonoid experiments: Cultured cells in 24-well multi-well plates were used for experimentation at 13 to 14 days in vitro. After washing with MEM, cultures were exposed to Aβ$_{25\text{-}35}$, Trolox, ascorbic acid, and various flavonoids for 24 or 48 hours.

Each row of the 24-well plates had 4 wells that were part of the same experiment. The four wells in the first row were treated with sham wash, NMDA (500 μM) treatment was given for full killing of neurons in the second row, and the third to the sixth rows were treated with drugs. The amount of each drug was 4 to 8 μL in each well with culture media. To evaluate the protective effects of these drugs on Aβ$_{25\text{-}35}$-induced injury, the authors treated the cultures with two drugs simultaneously.

3) Analysis of neuronal survival: Cell viability of the cultures was assessed according to morphological characteristics under phase-contrast microscopy.[30] The extent of neuronal injury was evaluated at the termination of Aβ ex-

posure for 24 hours and was based on the amount of lactate dehydrogenase (LDH) released into the medium at the end of the exposure period. Neuronal injury was quantified by measurement of LDH in the culture medium. LDH activity in the medium was measured as described by Koh and Choi.[31] For the measurement of LDH, 25 μL of culture medium was collected from each well of the 24-well plate and mixed with 125 μL of a buffer solution and 100 μL of 0.3 mg/mL NADH and then added to 30 μL of 22.7 mM pyruvate. We measured the density at 340 nm for 4 minutes by using a microplate reader (Molecular Device Co., USA). We used Sigma's control enzyme as the standard enzyme. Each condition was represented in three or four wells per experiment and was repeated 3 or 4 times in independent experiments. The measured values were calculated from 0 for the sham wash group to 100 for the full kill group and were reported as means±SEMs.

4) DPPH assay: An amount of 190 μL of 200 μM DPPH (1,1-diphenyl-2-picrylhydrazyl) with ethanol solution was added to 10 μL of DMSO and absorbance was measured immediately at 517 nm for the control reading.

For each of the flavonoid compounds, the sample volume was made uniformly to 10 μL by using ethanol and then 190 μL of 200 μM DPPH was added. Absorbance was measured after 30 minutes at 517 nm by using ethanol as the blank on a UV-visible spectrometer (Shimadzu, UV-1601, Japan). The concentration of each flavonoid compound was checked 3 times. The IC50 values for each compound and for the standard preparation were calculated. The DPPH free radical scavenging activity was calculated by using the following formula:

$$\%Scavenging = [(\text{Absorbance of control} - \text{Absorbance of test sample})/\text{Absorbance of control}] \times 100$$

5) Data analysis: Experiments were set up with 8 to 12 replicate samples. Results are expressed as mean±SEM values, and the agents' effects were statistically evaluated with analysis of variance (one-way ANOVA) followed by the Student-Newman-Keuls post hoc test. Statistical significance was considered at value of $p < 0.05$.

RESULTS

1. Aβ₂₅₋₃₅-induced neurotoxicity of cultured neurons

To evaluate the neurotoxicity of Aβ₂₅₋₃₅, primary cultures of mouse cortical cells containing neuronal and non-neuronal cells maintained for 13 to 14 days in vitro were exposed to Aβ₂₅₋₃₅ at each concentration (5, 10, 20, 40, and 80 μM) for 24 or 48 hours. Aβ₂₅₋₃₅ within a concentration range of 5 to 20 μM produced concentration-dependent neurotoxicity in cultured cortical neurons. Therefore, we used 20 μM Aβ₂₅₋₃₅ to induce neuronal cell damage in the following experiments.

Neuronal cell damage (Fig. 1A, B) was observed under a phase-contrast microscope after exposure to 20 μM Aβ₂₅₋₃₅ for 24 hours. On the basis of LDH measurements in the cul-
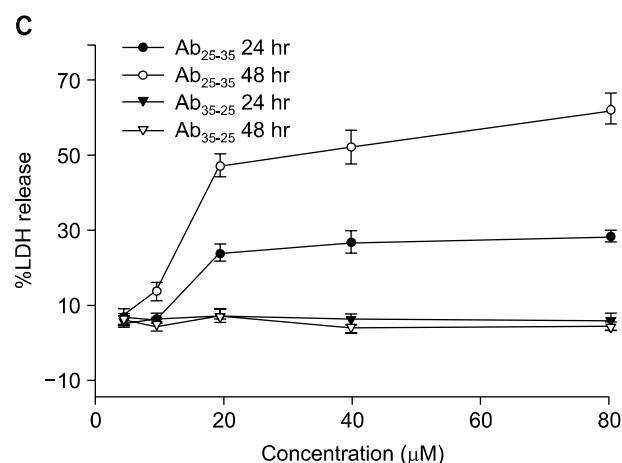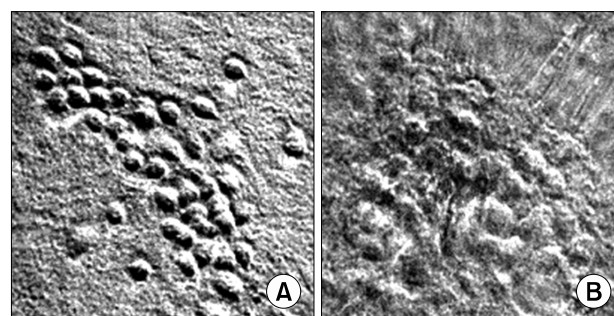

Fig. 1. Aβ₂₅₋₃₅-induced neuronal death in mouse cortical cultures. Phase-contrast photomicrographs from typical representative fields (200×field) of cells were taken after a 24-hour exposure to (A) sham wash or (B) 20 μM Aβ₂₅₋₃₅ showing neurotoxic action of 20 μM Aβ₂₅₋₃₅ in mouse cortical cultures (200×field). Treatment with Aβ₂₅₋₃₅ induced concentration- and exposure-time-dependent neuronal death in mixed cortical cultures (C). Each point and bars are the mean±SEM from 8-20 cultured wells (C).

ture media, at a concentration of 20 μM Aβ₂₅₋₃₅, approximately 20% or 50% of cells were damaged after exposure for 24 or 48 hours, respectively (Fig. 1C). Even when the concentration of Aβ₂₅₋₃₅ was increased to 40 and 80 μM, the LDH measurement was similar with that induced by 20 μM of Aβ₂₅₋₃₅ (Fig. 1C). Neurotoxicity was not induced by the reverse sequence of the Aβ₃₅₋₂₅ peptide.

2. Effects of antioxidants on Aβ₂₅₋₃₅-induced neuronal death

Free radical injury is one of the most common and important ways of inducing neuronal death. Aβ-induced neuronal death is also related to oxidative cell damage. To evaluate whether antioxidants such as Trolox and ascorbic acid could prevent Aβ₂₅₋₃₅-induced neuronal death in cultured neurons, the amount of LDH release in the culture media was measured after exposure of the cultured neurons to 20 μM Aβ₂₅₋₃₅ for 24 or 48 hours. On the basis of LDH measurements in the culture media, approximately 16±2.6% or 52±3.1% of cells were damaged by 20 μM Aβ₂₅₋₃₅ treatment for 24 hours (n=12) or 48 hours (n=12), respectively (Fig. 2A). Pretreatment with 100 μM Trolox reduced Aβ₂₅₋₃₅- in-

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duced LDH release to 4±3% (n=8) for 24 hours and 8±2.7% (n=8) for 48 hours, and 100 μM ascorbic acid reduced LDL release to 0±1.4% (n=8) for 24 hours and 5±2.5% (n=8) for 48 hours (Fig. 2).

3. Antioxidant activities of flavonoid compounds

According to the above data, the antioxidants Trolox and ascorbic acid strongly inhibited Aβ25-35-induced neuronal death. To test the antioxidant effects of flavonoid compounds in neuronal death, we used apigenin, baicalein, catechin, epicatechin, EGCG, kaempferol, luteolin, myricetin, quercetin, and luteolin as the flavonoid compounds. Scavenging of free radical DPPH is the basis of a common antioxidant assay. We therefore used the stable free 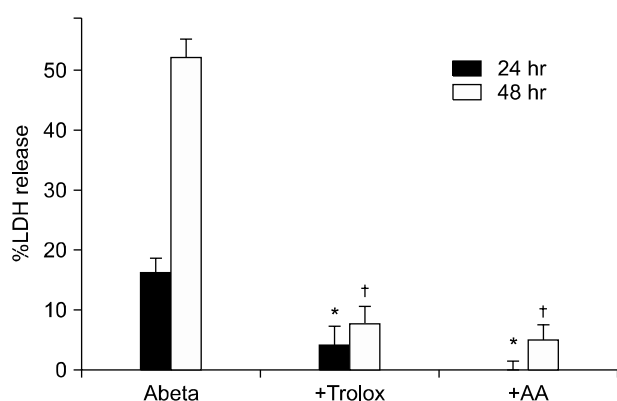radical DPPH to estimate the antioxidant activity of the flavonoid compounds. This method examines the use of the parameter IC_{50} (the concentration of substrate that causes 50% loss of the DPPH activity). All of the flavonoid compounds except for apigenin had strong antioxidant effects. Compared with the antioxidant effects of Trolox, kaempferol had similar effects, whereas the others had stronger antioxidant effects (Table 1).

4. Effects of flavonoids on Aβ25-35-induced neuronal death

Cultured neurons were treated with 20 μM Aβ25-35 for 24 or 48 hours and 30 μM of each flavonoid, and the amount of LDH release in the culture media was measured. Except for apigenin, all flavonoids inhibited Aβ25-35-induced neuronal death for 24 hours, but these protective effects were not maintained for 48 hours for baicalein, catechin, kaempferol, quercetin, and rutin (Table 2).

To determine whether the 3 flavonoids EGCG, luteolin, and myricetin could prevent Aβ25-35-induced neuronal death in cultured neurons, LDH release was measured after exposure of the neurons to 20 μM Aβ25-35 for 24 and 48 hours. To determine the flavonoid concentration for neuroprotective effects, we selected 3 flavonoids (EGCG, luteolin, myricetin) that inhibited Aβ25-35-induced neuronal death for 24 and 48 hours. Treatment with EGCG (3, 10, or 30 μM) significantly attenuated LDH release by Aβ25-35-induced neuronal death in a dose-dependent manner compared with the control (Fig. 3A). Compared with LDH release after treatment with 20 μM Aβ25-35 (21±2.3% at 24 hours, n=12, and 46±3.2% at 48 hours, n=12), pretreatment with 3, 10, or 30 μM EGCG reduced LDL release to 9±1.6%, 4±1.6%, and 1±04% at 24 hours (n=12) and to 20±2.5%, 8±1.6%, and 4±3.0% at 48 hours (n=12), respectively (Fig. 3A). Compared with LDH release after

FIG. 2. Effect of co-treatment with Trolox (100 μM) or ascorbic acid (AA, 100 μM) on 20 μM Aβ25-35-induced neuronal death at the end of 24 and 48 hours of exposure. Each bar is the mean±SEM from 8-16 cultured wells. *Significantly different from 24-hour-treated control group (p < 0.05). #Significantly different from 48-hour-treated control group (p < 0.05).

TABLE 1. Antioxidant ability of various flavonoids against the free radical DPPH

Flavonoids	Scavenging DPPH	
	% inhibition at 30 μM[a]	IC50 (μM)[b]
Apigenin	0.2±0.5	—
Baicalein	65±1.4	21.2
Catechin	79±0.9	15
EGCG	82±0.2	6.7
Epicatechin	75±1.1	19
Kaempferol	42±1.0	34.4
Luteolin	76±0.5	14.8
Myricetin	78±0.4	10.5
Quercetin	79±1.4	14.5
Rutin	67±3.9	17
Trolox	52±1.6	34.1

DPPH: 1,1-diphenyl-2-pycrylhydrazyl, EGCG: epigallocatechin gallate. [a]Represents the inhibition (%) of DPPH activity at the 30 μM concentration of each flavonoid. [b]Represents the concentration of substrate that causes 50% loss of the DPPH activity.

TABLE 2. Effects of some flavonoids on 20 μM Aβ25-35-induced neuronal death

Flavonoids (30 μM)	LDH release after exposure of 20 μM Aβ25-35	
	For 24 hours	For 48 hours
Control	21±1.1	46±1.9
Apigenin	23±2.0	44±2.8
Baicalcin	12±1.4*	49±2.4
Catechin	12±1.7*	37±3.6
Epicatechin	3±1.4*	13±2.4[†]
EGCG	1±0.4*	4±3.0[†]
Kaempferol	7±1.8*	46±2.3
Luteolin	1±1.4*	19±5.4[†]
Myricetin	1±1.6*	15±1.6[†]
Quercetin	10±2.0*	51±3.9
Rutin	7±1.1*	51±3.4

Aβ25-35: β-amyloid peptide fragment 25-35, LDH: lactate dehydrogenase, EGCG: epigallocatechin gallate. Numerals are the calculated percentage (mean±SE) of LDH release from 4×52 wells compared with complete neuronal death. * and [†] represent significant differences for 24 hours and 48 hours compared with the control group, respectively (p < 0.05).
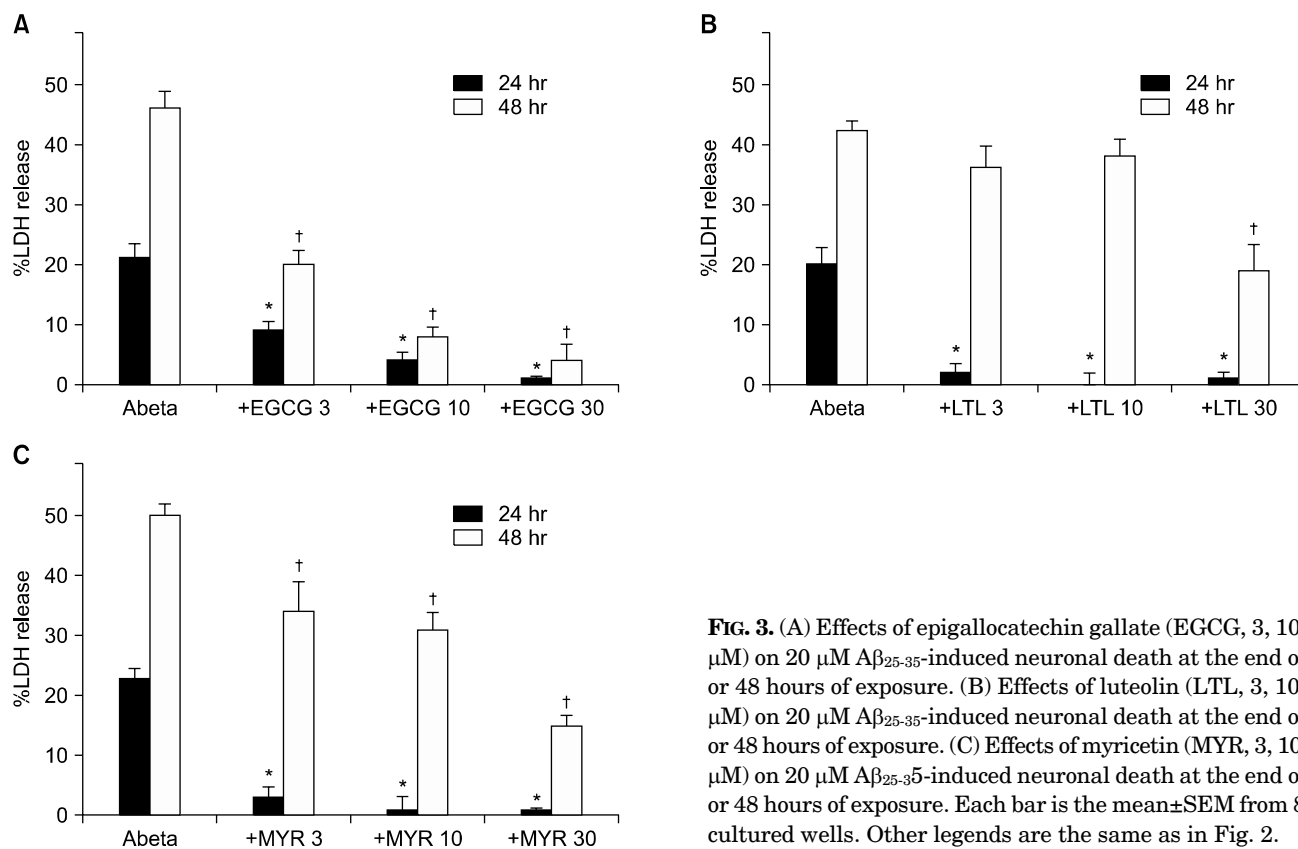
FIG. 3. (A) Effects of epigallocatechin gallate (EGCG, 3, 10, 30 μM) on 20 μM Aβ$_{25-35}$-induced neuronal death at the end of 24 or 48 hours of exposure. (B) Effects of luteolin (LTL, 3, 10, 30 μM) on 20 μM Aβ$_{25-35}$-induced neuronal death at the end of 24 or 48 hours of exposure. (C) Effects of myricetin (MYR, 3, 10, 30 μM) on 20 μM Aβ$_{25-3}$5-induced neuronal death at the end of 24 or 48 hours of exposure. Each bar is the mean±SEM from 8-12 cultured wells. Other legends are the same as in Fig. 2.

treatment with 20 μM Aβ$_{25-35}$ (20±2.7% at 24 hours, n=12, and 42±1.8% at 48 hours, n=12), pretreatment with 3, 10, or 30 μM luteolin reduced LDH release to 2±1.6%, 0±2.2%, and 1±1.4% at 24 hours (n=12) and to 36±3.4%, 38±2.6%, and 19±4.3% at 48 hours (n=12), respectively (Fig. 3B). At 24 hours, all concentrations of luteolin from 3 μM to 30 μM almost completely inhibited Aβ$_{25-35}$-induced neuronal death, but at 48 hours, only 30 μM luteolin significantly inhibited Aβ$_{25-35}$-induced neuronal death (Fig. 3B). In the experiment with myricetin, when cortical neurons were exposed to 20 μM Aβ$_{25-35}$ for 24 or 48 hours, the amounts of LDH release in the culture media were 23±1.7% (n=12) and 50±1.9% (n=12), respectively. Pretreatment with myricetin (3, 10, and 30 μM) reduced Aβ$_{25-35}$-induced LDH release to 3±1.8%, 1±2.1%, and 1±1.6% at 24 hours (n=12) and to 34±4.9%, 31±2.8%, and 15±1.6% at 48 hours (n=12), respectively (Fig. 3C).

DISCUSSION

The results of the present study showed that Aβ$_{25-35}$ treatment induced neuronal death that was dependent on dosage and exposure time, whereas the inverted amino acid sequence Aβ$_{35-25}$ failed to induce neuronal death. These results suggest that Aβ$_{25-35}$-induced neuronal death is specific. In addition, although increasing the concentration of Aβ$_{25-35}$ to at least 20 μM made a difference between a 24-hour time frame and a 48-hour time frame, there was no significant difference in the maximum effect

within the same time frame. Subsequently, these results imply that Aβ$_{25-35}$ acted selectively on only a fraction of the cultured neurons, which suggests that further investigation is needed.

In this study, treatment with the antioxidants Trolox and ascorbic acid noticeably blocked Aβ-induced neuronal death. In addition, among the 10 different flavonoid compounds tested in this study, the free radical scavenging inactive compound apigenin failed to block Aβ-induced neuronal death in DPPH scavenging-activity tests, whereas all other compounds significantly blocked Aβ-induced neuronal death after a 24-hour time frame. These results indicate that Aβ-induced neuronal death is associated with oxidative damage due to free radical formation. In fact, many studies suggest that oxidative damage is associated with Aβ-induced neuronal death.[23,24,32,33]

A search on free radical DPPH scavenging activity yielded a sequence of highest to lowest activity as follows: EGCG > myricetin > quercetin = luteolin = catechin ≥ rutin ≥ epicatechin ≥ baicalein > kaempferol = Trolox (Table 1). Such results indicate that the majority of the flavonoid compounds used in this study had a higher level of antioxidant activity than that of Trolox. Additionally, the inhibitory action of flavonoid compounds at a concentration of 30 μM on Aβ-induced neuronal death was (highest to lowest) as follows: EGCG = luteolin = myricetin = epicatechin > kaempferol = rutin ≥ quercetin ≥ baicalein = catechin (Table 2). Although there are some discrepancies between the rankings for antioxidant activity and those for the in-

hibitory activity of Aβ-induced neuronal death for some of these flavonoids, the finding that EGCG, the compound with the most antioxidant activity, also had the highest inhibitory activity on Aβ-induced neuronal death implies a possible correlation. In a previous study,[34] free radical DPPH scavenging activity was related with total phenolic content, which was related to cell viability in Aβ[25-35]-induced hippocampal neuronal death. Rice-Evans et al.[6] reported that upon measuring an alternate antioxidant activity marker such as Trolox-equivalent antioxidant activity (TEAC) in various flavonoid compounds, apigenin had greater antioxidant activity than kaempferol, and all other flavonoid compounds still had higher antioxidant activity than Trolox. The present study found that apigenin had negligible free radical DPPH scavenging activity as well as no influence on Aβ-induced neuronal death, which is not compatible with the findings of previous studies.[6,35] This result may be due to the use of free radical DPPH scavenging activity, which better reflects antioxidant activity on Aβ compared with TEAC. However, an explanation for why apigenin exhibited differing results for free radical DPPH scavenging activity versus TEAC remains to be investigated.

Among the flavonoid compounds used in this study, epicatechin, EGCG, luteolin, and myricetin showed the strongest inhibitory effects, which were maintained after a 48-hour time frame. The dose response tests of EGCG, luteolin, and myricetin showed that a low concentration of 3 to 10 μM significantly blocked Aβ-induced neuronal death. In particular, luteolin and myricetin had stronger inhibitory effects than did EGCG. On the basis of these results and considering that EGCG exhibited stronger inhibitory effects than luteolin or myricetin for free radical DPPH scavenging activity, the inhibitory effects of these compounds on Aβ-induced neuronal death may not always be proportional to antioxidant activity. Nevertheless, the inhibitory effect of EGCG on Aβ-induced neuronal death was substantially stronger after a 48-hour time frame. The difference between the levels of inhibition at 24 hours compared with 48 hours may be dependent on the chemical properties of the compounds, but this will require further inquiry.

Ginkgo biloba extract EGb 761 has been gaining attention for its effects in memory enhancement in the treatment of Alzheimer's disease.[36] The structure of EGb 761 as a polymer made up of basic flavonoids[37] and reports that this drug blocks Aβ-induced neuronal death in cultured neurons[23] suggest the potential of the various flavonoid compounds analyzed in this study to be developed as neuroprotectant agents. Currently, active studies are being done to develop compounds, including both flavonoids and antioxidants, for use as possible drugs to treat neurodegenerative diseases.[38-41]

In conclusion, this study was undertaken to analyze the effects of 10 different flavonoid compounds (apigenin, baicalein, catechin, epicatechin, EGCG, kaempferol, luteolin, myricetin, quercetin, and rutin) to search for a flavonoid compound that can be developed as a new drug to treat Aβ-induced neuronal death. Aβ[25-35]-induced neuronal death was dependent on dosage and exposure time. The results of this study indicated the presence of oxidative damage during Aβ-induced neuronal death and that flavonoid compounds with free radical scavenging activity can block Aβ-induced neuronal death. Finally, the results suggest that compounds that have strong inhibitory effects against Aβ[25-35]-induced neuronal death, such as epicatechin, EGCG, luteolin, and myricetin, have the potential to be developed into new drugs for the treatment of Alzheimer's disease.

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CONFLICT OF INTEREST STATEMENT

None declared.

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