JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Potent Inhibitory Effect of Flavonoids in *Scutellaria baicalensis* on Amyloid β Protein-Induced Neurotoxicity

HO JIN HEO,† DAE-OK KIM,† SOO JUNG CHOI,‡ DONG HOON SHIN,‡ AND CHANG YONG LEE*,†

Department of Food Science and Technology, Cornell University, Geneva, New York 14456, and Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea

The free radical scavenging activities of two major flavonoids (baicalein and baicalin) in *Scutellaria baicalensis* were determined. The antioxidant capacities of baicalein and baicalin were determined by the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)$^{\bullet-}$ scavenging assay and showed about 110 and 70% vitamin C equivalent antioxidant capacity, respectively. Because amyloid β (Aβ) protein is known to increase free radical production and lipid peroxidation in PC12 nerve cells, leading to apoptosis and cell death, treatment with baicalein and baicalin may result in the prevention of cellular damage by the Aβ-induced reactive oxygen species. We found that baicalein and baicalin resulted in a dose-dependent anti-Aβ toxicity by means of three different assays [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction, lactate dehydrogenase release, and trypan blue exclusion assays]. These results suggest that baicalein as well as baicalin can reduce the cytotoxicity of Aβ protein in PC12 cells, possibly by a reduction of oxidative stress, and these flavonoids may be useful in the chemoprevention of Alzheimer's disease.

KEYWORDS: Amyloid β protein; Alzheimer's disease; baicalein; baicalin; oxidative stress; vitamin C equivalent antioxidant capacity (VCEAC)

INTRODUCTION

A neurodegenerative disease such as Alzheimer's disease (AD) is closely related to oxidative stress. Amyloid β (Aβ) peptide is a 40−42 amino acid peptide and may be involved in the pathogenesis of AD (*1*). The Aβ peptide has been identified as a possible source of oxidative stress in the AD brain because it can acquire a free radical state contributing to its toxic effects (*2*). The generation of reactive oxygen species (ROS) and oxidative damage are believed to be involved in the pathogenesis of neurodegenerative disorders (*3*). It has been found that oxidative stress causes increased injury in neuronal cell nuclei and mitochondrial DNA (*4*) and that the activities of antioxidant enzymes decrease and the lipid peroxidation products increase in neurodegenerative brain tissue (*5*). Some brain regions of AD patients showed an increased sensitivity to oxygen free radicals; this could be due to a reduction in free radical defenses, an increase in free radical formation, or both (*6*).

The two major ROS produced by living tissue are the superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). Although H_2O_2 is not a free radical and has a limited reactivity, it can cross biological membranes, while $O_2^{\bullet-}$ can only move very slowly unless there is an anion channel through which it can travel (*7*). Thus, H_2O_2 is a more important contributor to pathological events than $O_2^{\bullet-}$. Aβ-induced cytotoxicity has been shown to be caused by the intracellular accumulation of H_2O_2, ultimately leading to the peroxidation of membrane lipids and to a cell death (*1*).

Many phenolic phytochemicals have antioxidative, anticarcinogenic, antimicrobial, antimutagenic, and antiinflammatory activities (*8*−*11*). Some phytochemicals including flavonoids in natural plant sources may reduce the risk of AD (*12*−*16*). However, there still remains a paucity of studies that have examined their role in brain functions such as AD. *Scutellaria baicalensis* is a traditional Asian herbal medicine, which has an antiinflammatory and antioxidant effect. It was reported that the active components in *S. baicalensis* include baicalin (5,6,7-trihydroxyflavone-7-*O*-β-D-glucopyranosideuronic acid) and its aglycone baicalein (5,6,7-trihydroxyflavone) (*17*). Baicalein and baicalin belong to the flavone subfamily of flavonoids. The antioxidant activities of these flavonoids have also been verified (*18*, *19*). However, the effects of these flavonoids on Aβ-induced neurotoxicity are relatively unknown. The purpose of this study was to investigate the possible protective effects of these flavonoids on Aβ-induced neuronal cell death with the sympathetic nerve pheochromocytoma cell line (PC12 cell).

MATERIALS AND METHODS

Materials. RPMI 1640 medium, fetal bovine serum, horse serum, penicillin, and streptomycin were obtained from Gibco BRL (Grand Island, NY). Sigma Chemical Co. (St. Louis, MO) supplied $Aβ_{25-35}$ and ABTS as the diammonium salt. $Aβ_{25-35}$ was dissolved in ddH_2O

* To whom correspondence should be addressed. Tel: +1-315-787-2271. Fax: +1-315-787-2284. E-mail: cyl1@cornell.edu.
† Cornell University.
‡ Korea University.

10.1021/jf049953x CCC: $27.50
Published on Web 06/05/2004

at a concentration of 1 mM and stored at −20 °C. The stock solution was diluted to the desirable concentrations immediately before use. Baicalin and baicalein were purchased from Aldrich Co. (St. Louis, MO). AAPH was obtained from Wako Chemicals USA, Inc. (Richmond, VA). Ascorbic acid was purchased from Fisher Scientific (Pittsburgh, PA). All other chemicals used were analytical grade.

ABTS Radical Scavenging Assay. Blue-green ABTS radicals were used to evaluate the antioxidant capacity of baicalin and baicalein (*20*). A radical initiator, 1.0 mM AAPH, was added to 2.5 mM ABTS in phosphate-buffered saline (PBS; pH 7.4; 0.1 M K_2HPO_4/KH_2PO_4 buffer; 150 mM NaCl). The mixed solution was heated in a water bath at 68 °C. The resulting blue-green ABTS radical solution was adjusted to an absorbance of 0.650 ± 0.020 at 734 nm with additional PBS. Twenty microliters of sample was added to 980 μL of the ABTS radical solution. The mixture was incubated in a 37 °C water bath under restricted light. A control (20 μL of 50% methanol and 980 μL of ABTS radical solution) was run with each series of samples. The reduction of absorbance at 734 nm was measured at 10 min. The ABTS radical, showing its characteristic blue-green color in its odd electron state, loses color when the electron from vitamin C, baicalein, and baicalin pairs its unpaired electron. The radical stock solution was prepared fresh daily.

Cells. PC12 cells were cultured and maintained as previously described (*21*). The PC12 cell line was derived from a transplantable rat pheochromocytoma. The cells respond reversibly to nerve growth factor by the induction of the neuronal phenotype growth medium consisting of RPMI 1640, 10% heat-inactivated horse serum, 5% fetal bovine serum, 50 units/mL penicillin, and 100 μg/mL streptomycin. The cultures were maintained in the 37 °C incubator with water-saturated and 5% CO_2. PC12 cells were passaged when the culture was 80−90% confluent, dislodged from the surface of the culture dish (100 mm × 20 mm), and dispersed into single cells by repeated and forceful triturating of the culture medium directly onto the cells. The cells were subcultured once a week in the split ratio of 1:3 or 1:4.

MTT Assay. The MTT reduction assay was performed as described previously (*22*, *23*). Briefly, the cells were incubated with 0.25 mg MTT/mL for 0.5−6 h at 37 °C, and the reaction was stopped by adding a solution containing 50% dimethylformide and 20% sodium dodesyl sulfate (pH 4.8). The amount of MTT formazan product was determined by measuring the absorbance using a microplate reader (Bio-Rad, Hercules, CA) at a test wavelength of 570 nm and a reference wavelength of 630 nm.

LDH Assay. The damage of the plasma membrane was evaluated by measuring the amount of the intracellular enzyme LDH released into the medium. Fifty microliters of culture supernatants was collected from each well, and the LDH activities were determined with a colorimetric LDH assay kit (Sigma). The total cellular LDH activity was determined by solubilizing the cell with 0.2% Triton X-100 (*24*) and set as 100%.

Trypan Blue Test for Cell Viability. Cell viability was assessed by trypan blue dye exclusion prior to all treatments. Viable cells maintained membrane integrity and did not allow trypan blue dye to pass through the cell membrane. Cells with a compromised cell membrane appeared blue due to their accumulation of dye and were counted as dead. At least 600 cells were counted in four different fields, and the number of viable cells was calculated as a percent of the total cell population. PC12 cells with ≥98.0% viability were employed in all treatments (*25*).

Statistical Analysis. All data were expressed as means ± SD. Statistical analysis was performed by Student's *t*-test. Statistical comparisons within the same group were performed for paired observations. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The antioxidant effect of baicalein and baicalin was previously reported (*18*, *19*). In this study, their protective effects against Aβ-induced oxidative stress were examined. The antioxidant capacities of baicalein and baicalin, as determined by scavenging ABTS radical chromogens, are presented in **Figure 1**. AAPH,

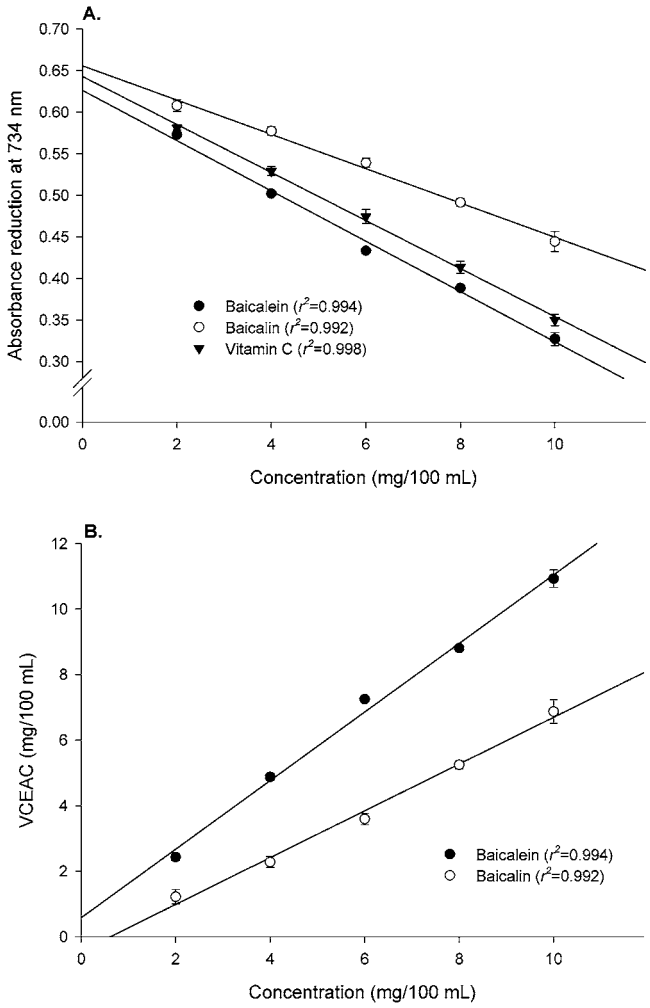

Figure 1. ABTS radical scavenging assay of baicalein and baicalin from *S. baicalensis*. (**A**) Relationship between vitamin C or two flavonoids and absorbance reduction of free blue-green ABTS radical at 734 nm. (**B**) Conversion of antioxidant capacity of two flavonoids into VCEAC by ABTS assay. The data are displayed with means ± SD (bars) of three replications.

a thermolabile water soluble radical initiator, oxidized $ABTS^{2-}$ to ABTS radical anion (*26*). The generation of the ABTS radical anion before the addition of antioxidants was reported to eliminate the interference of compounds affecting radical production (*27*). Vitamin C standard curves that relate the concentration of vitamin C and the amount of absorbance reduction caused by vitamin C were obtained using the ABTS radical scavenging assay (**Figure 1A**). The absorbance at 734 nm of baicalein and baicalin was also measured at various concentrations by the ABTS assay. The calculation of VCEAC of each compound at the various concentration levels was made using vitamin C standard curve (**Figure 1B**). The stable ABTS radical scavenging activity of baicalein and baicalein was expressed as mg/100 mL of VCEAC in 10 min. All tested samples were replicated three times. The calibration curve showed a linear relationship ($r^2 = 0.998$) between vitamin C concentration and absorbance reduction at 734 nm (**Figure 1A**). The first order of linear regression in relation to the absorbance reduction vs various concentrations of baicalein and baicalin was attained using the correlation coefficient (r^2), which was found to be close to that of vitamin C (**Figure 1A**). The antioxidant capacity of two flavonoids showed an apparent first-order relationship with VCEAC (**Figure 1B**). From **Figure 1B**, the antioxidant capacities of baicalein and baicalin can easily

be converted to VCEAC on a weight basis. As the plotted lines of baicalin in **Figure 1B** are below the tangential line of 45°, baicalin shows a lower antioxidant capacity than vitamin C whereas baicalein has a higher antioxidant potential than vitamin C. The antioxidant capacities of baicalein and baicalin were 11.39 and 6.87 mg/100 mL VCEAC, respectively. The hydroxyl groups on the A ring of flavone baicalein are apparently attributable to its antioxidant capacity. On the other hand, there was no antioxidant capacity in the flavone skeleton alone, which possesses no hydroxyl group on its structure (data not shown). Heijnen et al. (*28*) showed the antioxidant activities of dozens of flavonoids and came to the conclusion that glycosylation of flavonoids reduced their activity when compared to the corresponding aglycons. The overall relative antioxidant capacities of baicalein, baicalein, and vitamin C in the ABTS assay were in a decreasing order as follows: baicalein > vitamin C > baicalin.

Oxidative stress reflects a situation wherein ROS, such as free radicals and their products, are in excess over the antioxidant defense systems (*29*). The link between the amyloid deposits and oxidative stress in the AD brain is not readily apparent due to the fact that studies of postmortem tissue cannot reveal whether these deposits are the byproducts of neurodegeneration or precede the degenerative process. However, there is increasing evidence showing that Aβ itself is associated with oxidative stress. Several markers of excess oxidative stress such as accumulation of oxidized products (protein carbonyls from protein oxidation and aldehydes and isoprostanes from lipid peroxidation) serve to establish the direct role of Aβ in the oxidative damage associated with AD (*16*, *29*). To evaluate Aβ oxidative injury properly, it is important to employ an appropriate method for quantitating cell viability. Because the dye, MTT, is known to be converted into a purple formazan by the redox activity of living cells, the MTT reduction assay has been widely used for measuring cell viability (*22*, *23*). The cellular reactions involved in this reduction are not completely understood today. The involvement of active mitochondria, namely, the mitochondrial succinate dehydrogenase system, has been discussed (*30*). PC12 cells were treated with Aβ_{25-35} (50 μM) for 2 h, and the effect of Aβ-induced cytotoxicity was evaluated with the MTT assay. Aβ_{25-35} caused a decrease in cell viability (about 47%), but the pretreatment of cells with increasing concentrations of baicalein and baicalin inhibited Aβ-induced neurotoxicity (**Figure 2**). The cell protection effect of vitamin C at 100 μM against Aβ-induced neurotoxicity was less than that of the flavonoids at the corresponding concentration. Therefore, these results indicate that PC12 cell protection by the two flavonoids, antioxidants, is partially due to the mitochondrial protection mechanisms.

The membrane bilayer resident phospholipid unsaturated fatty acids (PUFAs) are especially vulnerable to free radical attack. Lipid peroxidation can lead to changes in the membrane fluidity; formation of conjugated dienes, 4-hydroxynonenal, acrolein, and isoprostanes; the release of free fatty acids; and a consequent decrease in levels of PUFAs, etc. (*31*, *32*). Because the neuronal plasma membrane is sensitive to oxidative stress (*1*, *31*, *32*), the toxic effect of Aβ_{25-35} was evaluated by the LDH assay, measuring the activity of this stable enzyme released into the medium from dead cells. A quantitative analysis of LDH activity can determine what percentage of cells is dead. Treatment with Aβ_{25-35} caused an increase in LDH release. Pretreatment with baicalein and baicalin inhibited LDH release in PC12 cells (**Figure 3**). To confirm if two flavonoids inhibit the Aβ-induced membrane damage, the trypan blue exclusion assay was also utilized. Viable cells maintained membrane integrity and did not allow trypan blue dye to pass through the cell membrane. As shown in **Table 1**, Aβ-induced oxidative stress increased plasma membrane damage. The PC12 cell plasma membrane was damaged by Aβ-induced oxidative stress. The Aβ-induced membrane damage was reduced by baicalein and baicalin. These results indicated that the effect of Aβ detected by the MTT assay corresponded with a decrease in cell viability and that Aβ-induced neurotoxicity was developed by plasma membrane

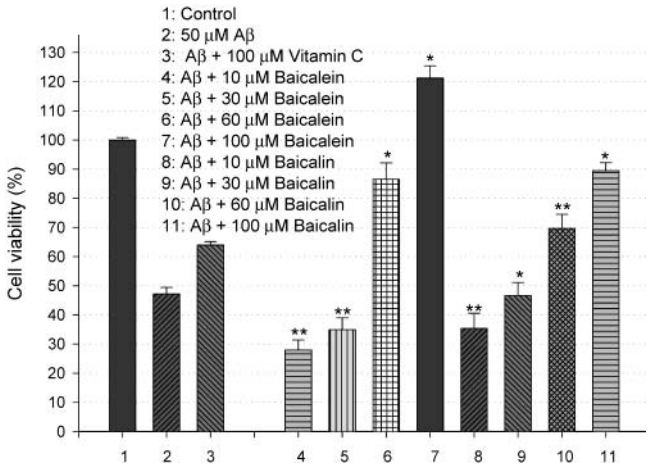

Figure 2. Effect of baicalein, baicalin, or vitamin C on PC12 cell viability determined in the presence and absence of Aβ. PC12 cells were treated for 10 min with various concentrations of the indicated compounds. The cells were then washed and treated with 50 μM Aβ_{25-35} for 2 h. The levels of cell viability were measured using the MTT assay as described in the Materials and Methods. The cell viability was not changed by vitamin C or two flavonoids (data not shown). Results shown are means ± SD (n = 3). A significant difference (*P < 0.01 or **P < 0.05 vs vitamin C treatment group) was observed on the Aβ-induced cell death.

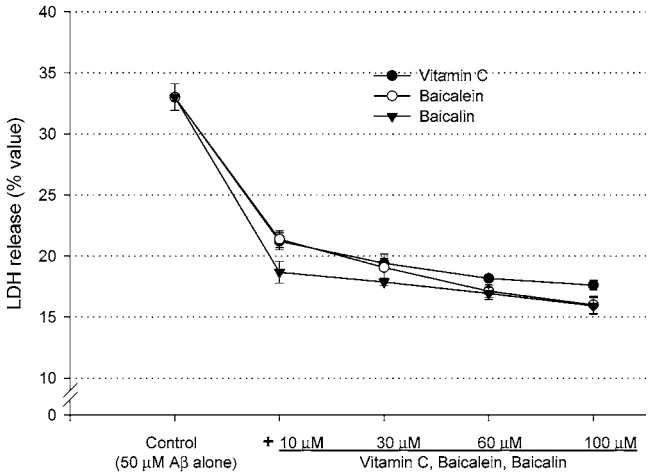

Figure 3. Preventive effects of baicalein, baicalin, or vitamin C on Aβ-induced lipid peroxidation in PC12 cells. PC12 cells were treated for 10 min with various concentrations of the indicated compounds. The cells were treated with Aβ_{25-35} (50 μM) for 2 h. The LDH activity in culture supernatants was measured with a colorimetric LDH assay kit. The basal and total LDH activities were determined in intact cells and cells solubilized with 0.2% Triton X-100, respectively, and the LDH release was calculated as [(sample LDH − basal LDH)/(total LDH − basal LDH)] × 100 (%). All data are represented as the means ± SD (n = 3). Values were obtained from three separate cultures. Statistical analysis indicated that the influence of the compounds used had a significant effect on the Aβ-induced membrane toxicity (LDH release) (P < 0.05).

Table 1. Inhibition of Aβ Toxicity in PC12 Cells after Pretreatment with Baicalein and Baicalin As Assessed by Trypan Blue Exclusion Staining Followed by Cell Counting

	cell viability (%)
control cells[a]	100
50 μM Aβ alone	38 ± 1.3
+ 100 μM vitamin C	59 ± 3.1
+ 10 μM baicalein	36 ± 2.4[c]
+ 30 μM baicalein	50 ± 3.2[c]
+ 60 μM baicalein	59 ± 4.4[c]
+ 100 μM baicalein	74 ± 4.3[b]
+ 10 μM baicalin	41 ± 2.8[b]
+ 30 μM baicalin	49 ± 2.7[c]
+ 60 μM baicalin	64 ± 5.9[b]
+ 100 μM baicalin	84 ± 2.0[c]

[a] PC12 cells were plated at a low density in a 24 well plate. The cells were incubated with these flavonoids for 10 min before the addition of 50 μM Aβ protein. The cultures were observed after an additional 2 h, and trypan blue exclusion staining was performed. Data are presented as means ± SD for one representative triplicate determination and are expressed as the percent survival as compared to the corresponding controls. The viability of untreated control cells was defined as 100%. [b] $P < 0.01$ vs vitamin C treatment group. [c] $P < 0.05$ vs vitamin C treatment group.

damage. In this study, baicalein and baicalin inhibited Aβ-induced membrane damage and neurotoxicity in a dose-dependent pattern.

Lipoxygenase is an important mediator of the inflammatory process, and chronic inflammation occurs in AD pathogenesis (*33*). Many lipoxygenase inhibitors can inhibit lipid peroxidation, and some inhibitors of lipid peroxidation such as quercetin have also been shown to inhibit lipoxygenase (*34*). Baicalein was previously reported to be a potently selective 12-lipoxygenase inhibitor (*35*) and showed physiological effects related to arachidonic acid metabolism (*36*). Baicalein have also been shown to block Aβ-induced 12-lipoxygenase (*33*). Therefore, the explanation for the protection effect of baicalein and baicalin in this study may be due to two factors: one is the direct scavenge of free radicals produced by Aβ, and the other factor is their action as 12-lipoxygenase inhibitors protecting the cell membrane from the damage induced by Aβ-induced oxidative stress. In conclusion, baicalein and baicalein could protect neuronal cells from Aβ-induced oxidative stress and might be used to ameliorate chemopreventive agents in AD.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane)dihydrochloride; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); Aβ, amyloid β; AD, Alzheimer's disease; APP, amyloid precursor protein; ddH_2O, distilled deionized water; ROS, reactive oxygen species; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LDH, lactate dehydrogenase; PUFAs, phospholipid unsaturated fatty acids; VCEAC, vitamin C equivalent antioxidant capacity.

LITERATURE CITED

(1) Behl, C.; Davis, J. B.; Lesley, R.; Schubert, D. Hydrogen peroxide mediates amyloid β protein toxicity. *Cell* **1994**, *77*, 817−827.

(2) Pappolla, M. A.; Chyan, Y. J.; Omar, R. A.; Hsiao, K.; Perry, G.; Smith, M. A.; Bozner, P. Evidence of oxidative stress and in vivo neurotoxicity of beta-amyloid in a transgenic mouse model of Alzheimer's disease: a chronic oxidative paradigm for testing anti-oxidant therapies in vivo. *Am. J. Pathol*. **1998**, *152*, 871−877.

(3) Coyle, J. T.; Puttfarcken, P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* **1993**, *262*, 689−695.

(4) Mecocci, P.; MacGarvey, U.; Beal, M. F. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol*. **1994**, *36*, 747−751.

(5) Markesbery, W. R. Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biol. Med.* **1997**, *23*, 134−147.

(6) Marcus, D. L.; Thomas, C.; Rodriguez, C.; Simberkoff, K.; Tsai, J. S.; Strafaci, J. A.; Freedman, M. L. Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. *Exp. Neurol.* **1998**, *150*, 40−44.

(7) Multhaup, G.; Ruppert, T.; Schlicksupp, A.; Hesse, L.; Beher, D.; Masters, C. L.; Beyreuther, K. Reactive oxygen species and Alzheimer's disease. *Biochem. Pharmacol.* **1997**, *54*, 533−539.

(8) Cao, Y.; Cao, R. Angiogenesis inhibited by drinking tea. *Nature* **1999**, *398*, 381.

(9) Eberhardt, M. V.; Lee, C. Y.; Liu, R. H. Antioxidant activity of fresh apples. *Nature* **2000**, *405*, 903−904.

(10) Ito, A.; Shamon, L. A.; Yu, B.; Mata-Greenwood, E.; Lee, S. K.; van Breemen, R. B.; Mehta, R. G.; Farnsworth, N. R.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. Antimutagenic constituents of *Casimiroa edulis* with potential cancer chemopreventive activity. *J. Agric. Food Chem*. **1998**, *46*, 3509−3516.

(11) Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M. Antiproliferative effects of the readily extractable fractions prepared from various citrus juices on several cancer cell lines. *J. Agric. Food Chem.* **1999**, *47*, 2509−2512.

(12) Ishige, K.; Schubert, D.; Sagara, Y. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. *Free Radical Biol. Med*. **2001**, *30*, 433−446.

(13) Commenges, D.; Scott, V.; Renaud, S.; Jacqmin-Gadda, H.; Barberger-Gateau, P. Intake flavonoids and risk of dementia. *Eur. J. Epidemiol*. **2000**, *16*, 357−363.

(14) Grundman, M.; Delaney, P. Antioxidant strategies for Alzheimer's disease. *Proc. Nutr. Soc*. **2002**, *61*, 191−202.

(15) Youdim, K. A.; Joseph, J. A. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. *Free Radical Biol. Med*. **2001**, *30*, 583−594.

(16) Prasad, K. N.; Cole, W. C.; Prasad, C. Risk factors for Alzheimer's disease: role of multiple antioxidants, nonsteroidal antiinflammatory and cholinergic agents alone or in combination in prevention and treatment. *J. Am. Coll. Nutr*. **2002**, *21*, 506−522.

(17) Nakamura, N.; Hayasaka, S.; Zhang, X. Y.; Nagaki, Y.; Matsumoto, M.; Hayasaka, Y.; Terasawa, K. Effects of baicalein, baicalein, and wogonin on interleukin-6 and interleukin-8 expression, and nuclear factor-κb binding activities induced by interleukin-1β in human retinal pigment epitherial cell line. *Exp. Eye Res*. **2003**, *77*, 195−202.

(18) Gao, D.; Sakurai, K.; Katoh, M.; Chen, J.; Ogiso, T. Inhibition of microsomal lipid peroxidation by baicalein: a possible formation of an iron-baicalein complex. *Biochem. Mol. Biol. Int.* **1996**, *39*, 215−225.

(19) Gao, Z.; Huang, K.; Xu, H. Protective effects of flavonoids in the roots of *Scutellaria baicalensis* georgi against hydrogen peroxide-induced oxidative stress in HS-SY5Y cells. *Pharmacol. Res.* **2001**, *43*, 173−178.

(20) Kim, D.-O.; Lee, K. W.; Lee, H. J.; Lee, C. Y. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J. Agric. Food Chem.* **2002**, *50*, 3713−3717.

(21) Heo, H. J.; Hong, S. C.; Cho, H. Y.; Hong, B. S.; Kim, H. K.; Kim, E. K.; Shin, D. H. Inhibitory effect of zeatin, isolated from *Fiatoua villosa*, on acetylcholinesterase activity from PC12 cells. *Mol. Cells* **2002**, *13*, 113−117.

(22) Abe, K.; Kimura, H. Amyloid β toxicity consists of a Ca^{2+} independent early phase and a Ca^{2+} dependent late phase. *J. Neurochem*. **1996**, *67*, 2074−2078.

(23) Kato, M.; Saito, H.; Abe, K. Nanomolar amyloid β protein-induced inhibition of cellular redox activity in cultured astrocytes. *J. Neurochem*. **1997**, *68*, 1889−1895.

(24) Heo, H. J.; Cho, H. Y.; Hong, B. S.; Kim, H. K.; Kim, E. K.; Kim, B. K.; Shin, D. H. Protective effect of 4′,5-dihydroxy-3′,6,7-trimethoxyflavone from *Artemisia asiatica* against Aβ-induced oxidative stress in PC12 cells. *Amyloid* **2001**, *8*, 194−201.

(25) Ray, S. K.; Fidan, M.; Nowak, M. W.; Wilford, G. G.; Hogan, E. L.; Banik, N. L. Oxidative stress and Ca^{2+} influx upregulate calpain and induce apoptosis in PC12 cells. *Brain Res.* **2000**, *852*, 326−334.

(26) Scott, S. L.; Chen, W. J.; Bakac, A.; Espenson, J. H. Spectroscopic parameters, electrode potentials, acid ionization constants, and electron exchange rates of the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radicals and ions. *J. Phys. Chem.* **1993**, *97*, 6710−6714.

(27) van den Berg, R.; Haenen, G. R. M. M.; van den Berg, H.; Bast, A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* **1999**, *66*, 511−517.

(28) Heijnen, C. G. M.; Haenen, G. R. M. M.; Oostveen, R. M.; Stalpers, E. M.; Bast, A. Protection of flavonoids against lipid peroxidation: the structure activity relationship revisited. *Free Radical Biol. Med.* **2002**, *36*, 575−581.

(29) Ferrari, C. K. B.; Torres, E. A. F. S. Biochemical pharmacology of functional foods and prevention of chronic disease of aging. *Biomed. Pharmacother.* **2003**, *57*, 251−260.

(30) Carmichael, J.; De Graff, W. G.; Gadzar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of tetrazolium-based semi-automated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res.* **1987**, *46*, 936−941.

(31) Morrow, J. D.; Roberts, L. J. The isoprostanes: unique bioactive products of lipid peroxidation. *Prog. Lipid Res.* **1997**, *36*, 1−21.

(32) Tsai, A. L.; Palmer, G.; Xiao, G.; Swinney, D. C.; Kalmacz, R. J. Structural characterization of arachidonyl radicals formed by prostaglandin H synthase-2 and prostaglandin H synthase-1 reconstituted with mangano protoporphyrin IX. *J. Biol. Chem.* **1998**, *273*, 3888−3894.

(33) Antony, L.; Françoise, E.; William, R.; Didier, P. Baicalein protects cortical neurons from β-amyloid (25-35) induced toxicity. *Neuroreport* **2001**, *12*, 2199−2202.

(34) Takahama, U. Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin:mechanisms of antioxidative function. *Phytochemistry* **1985**, *24*, 1443−1446.

(35) Nadler, J. L.; Natarajan, R.; Stern, N. Specific action of the lipoxygenase pathway in mediating angiotensin II-induced aldosterone synthesis in isolated adrenal glomerulosa cells. *J. Clin. Invest.* **1987**, *80*, 1763−1769.

(36) Normandin, M.; Gagne, J.; Bernard, J.; Elie, R.; Miceli, D.; Baudry, M.; Massicotte, G. Involvement of the 12-lipoxygenase pathway of arachidonic acid metabolism in homosynaptic long-term depression of the rat hippocampus. *Brain Res.* **1996**, *730*, 40−46.

Received for review January 8, 2004. Revised manuscript received April 16, 2004. Accepted May 2, 2004. This work was supported by the Postdoctoral Fellowship Program of the Korea Science & Engineering Foundation (2003) for H.J.H. at Cornell University.

JF049953X