J Pharmacol Sci **98**, 185 – 189 (2005)

Journal of Pharmacological Sciences

Short Communication

Baicalein Prevents 6-Hydroxydopamine-Induced Dopaminergic Dysfunction and Lipid Peroxidation in Mice

Heh-In Im[1], Wan Seok Joo[2], Eunjoo Nam[1], Eun-sun Lee[1], Yu-jin Hwang[1], and Yong Sik Kim[1,*]

[1]*Department of Pharmacology, College of Medicine and Neuroscience Research Institute, Medical Research Center, Seoul National University, Seoul 110-799, Korea*
[2]*Division of Biologics Evaluation, Korea Food and Drug Administration, Seoul 122-704, Korea*

Received January 11, 2005; Accepted April 28, 2005

Abstract. The effects of baicalein on 6-hydroxydopamine (6-OHDA)-induced neurotoxicity were evaluated. Intracerebroventricularly (i.c.v.) injection of 6-OHDA was done to young mice. Baicalein was administered intraperitoneally 30 min before and 90 min after i.c.v. injection. Animals received further injection of bacalein daily for 3 consecutive days. Rotarod performance was assessed, tyrosine hydroxylase (TH) Western blotting was performed, and dopamine (DA) levels and peroxidation were determined. High dose of baicalein effectively improved rotarod performance and prevented the reduction of striatal DA levels and TH contents in the striatum and subtantia nigra (SN). In addition, lipid peroxidation level was decreased by baicalein at 3 and 7 days after 6-OHDA injection. These results showed that baicalein effectively prevents the 6-OHDA-induced dopaminergic dysfunction through an antioxidative action.

Keywords: 6-hydroxydopamine, baicalein, tyrosine hydroxylase

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by movement dysfunction, most notably hypokinesia, rigidity, tremor, and postural abnormalities. Most PD motor symptoms are attributed to dopaminergic neuron loss in the substantia nigra pars compacta (SNpc), resulting in striatal dopamine (DA) deficiency (1). Although PD etiology remains obscure, current evidence points to the presence of ongoing oxidative stress and reactive oxygen species (ROS) generation (1 – 3). There is an increasing amount of evidence that the neurotoxicity of 6-OHDA is mainly due to the oxidation of 6-OHDA, resulting in the generation of cytotoxic free radicals, which are believed to play a pivotal role in the degeneration of the nigrostriatal dopaminergic system (4). Thus oxidative damage caused by 6-OHDA can be prevented by the use of a variety of antioxidants (5, 6).

Flavonoids are a group of polyphenolic compounds of plant origin. They exhibit a variety of biological activities. Flavonoids are known to scavenge free radicals (7), chelate metal ions (8), and increase the expression of antioxidant proteins (9). Baicalein, one of the major flavonoids present in the root of *Scutellaria baicalensis*, has antioxidant and free radical scavenging effects (7). Baicalein effectively scavenged hydroxyl radicals and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and inhibited both enzymatically- and non-enzymatically-induced mitochondrial lipid peroxidation (10). Therefore, in the present study, the protective effects of baicalein on dopaminergic dysfunction in the 6-OHDA-induced PD model were evaluated.

Desipramine (25 mg/kg) was intraperitoneally administered to male ICR mice (26 to 28 g) 1 h before injection of 6-OHDA to block noradrenaline reuptake. Fifty micrograms of 6-OHDA (10 μg/μl with 0.2 mg/ml L-ascorbic acid; Sigma, St. Louis, MO, USA) or the same volume of the vehicle (L-ascorbic acid) was injected into the cerebral ventricles of each mouse. Baicalein (25 and 50 mg/kg, i.p.; Aldrich, St. Louis, MO, USA) dissolved in 5% DMSO was administered 30 min before and 90 min after intracerebroventricular (i.c.v.) injection. Following i.c.v. injection of the 6-OHDA, mice received daily doses of baicalein for 3 consecutive days. Sham (L-ascorbic acid)-injected mice received baicalein or 5% DMSO (instead of baicalein) and 6-OHDA-injected mice received 5% DMSO. Three or seven days after

*Corresponding author. FAX: +82-2-745-7996
E-mail: kimysu@plaza.snu.ac.kr

i.c.v. injection, striatal and SN tissues were collected.

The accelerating rotarod test was performed with ACCELER (ROTA-ROD for mice 7650 by Ugo Basile, Varese, Italy). After adaptation of fixed speed (20 rpm) for 5 min, the mice were placed on the horizontal plastic rod rotating at an initial speed of 5 rpm, and the rotational velocity of the rod was linearly increased from 5 to 50 rpm within 10 min. The time that each mouse was able to maintain its balance walking on the top of the rod was measured.

Tyrosine hydroxylase (TH) expression was examined by Western blot analysis. Tissues were sonicated with homogenizing buffer [50 mM Tris (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, and 1% SDS]. An equal amount of protein was separated by 12% SDS-polyacrylamide gel electrophoresis and transferred to a Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The membrane was incubated sequentially with TH primary antibody (Chemicon, Temecula, CA, USA) and horseradish peroxidase-conjugated antimouse IgG (Vector labs, Burlingame, CA, USA) followed by ECL detection (Amersham Pharmacia Biotech).

High performance liquid chromatography (HPLC) with electrochemical detector was used to determine striatal levels of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) as described by Ara et al. (11) with the following modifications: Dihydroxybenzylamine (DHBA) was added as an internal standard. Ten microliters of the samples was injected onto a μ-Bondapak C18 (3.9 × 300 mm column; Waters, Milford, MA, USA).

For measurement of malondialdehyde (MDA), 3 or 7 days after 6-OHDA lesion, tissues were diluted with homogenizing buffer (10 mM K_2HPO_4, KH_2PO_4, 30 mM KCl, 1 mM EDTA, pH 7.4). Homogenated tissue (300 μl) was mixed with cocktail solution (8.1% SDS, 20% acetic acid, 0.67% thiobarbituric acid) and shaken vigorously, boiled for 1 h, and then cooled with tap water. After centrifuging the solution, optical density of malondialdehyde in supernatant was measured at 532-nm wavelength. All data were presented as the mean ± S.E.M. Statistical comparison between different treatments was done by one-way analysis of variance (ANOVA) with Duncan's multiple test. Significance was taken as $P<0.05$.

In the rotarod performance test, the latency time to fall off from the rod was reduced in 6-OHDA-injected mice (61.5% of sham-treated mice), while there was no difference in motor performance between 6-OHDA-treated without and with 5% DMSO. Sham-treated only was not different from that with baicalein (data not shown) or with 5% DMSO as well. Therefore, we injected all experimental mice with vehicle (sham/vehicle, 6-OHDA/vehicle). However, the latency time to fall off was increased to 65.8% and 82.1% of sham-treated mice by 25 and 50 mg/kg baicalein treatment, respectively (Fig. 1). These results indicate that high dose of baicalein treatment showed a significant improvement in rotarod performance from 6-OHDA-induced motor deficits.

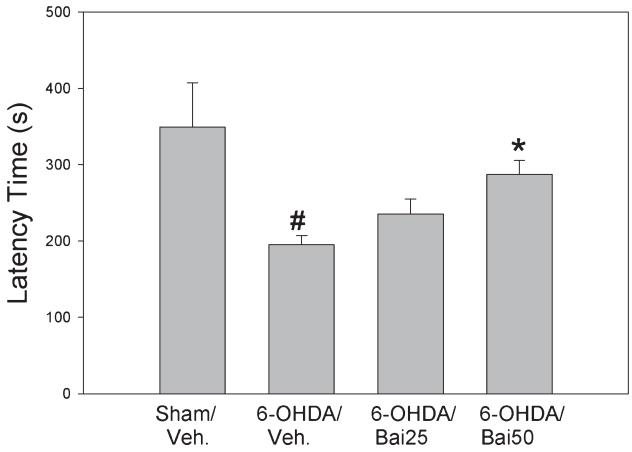

Fig. 1. Effects of baicalein on the rota-rod test in 6-hydroxydopamine (6-OHDA)-injected mice. Seven days after 6-OHDA injection, latency time to fall off was recorded maximally for 600 s. Eight to ten mice were used for each experimental group. Data were expressed as the mean ± S.E.M. *$P<0.05$: 6-OHDA/vehicle *vs* 6-OHDA/baicalein, $^{\#}P<0.05$: Sham/vehicle *vs* 6-OHDA/vehicle.

TH contents in the striatum and SN were determined by Western blotting. Striatal TH content was markedly decreased by 6-OHDA injection to 43.1 ± 3.1% of sham treated mice (Fig. 2A). However, the treatment with baicalein at 25 and 50 mg/kg significantly increased striatal TH contents to 53.0 ± 2.5% and 94.8 ± 1.3%, respectively, of sham-treated mice. Also TH expression in SN was significantly reduced to 26.1 ± 4.1% of sham-treated mice at 7 days after 6-OHDA injection compared with the sham-treated group (Fig. 2B). Baicalein treatment at 25 and 50 mg/kg markedly increased TH contents in SN to 70.5 ± 3.4% and 90.1 ± 1.1%, respectively, of sham-treated mice.

The levels of striatal DA and DOPAC were determined after 6-OHDA injection. Striatal DA levels were significantly reduced to 44.7 ± 5.8% of sham-treated mice by i.c.v. injection of 6-OHDA. However, baicalein treatment at 25 and 50 mg/kg dose-dependently increased striatal DA levels to 68.5 ± 8.2% and 88.9 ± 2.4%, respectively, of sham-treated mice (Fig. 2C). When intact (no i.c.v. injection) mice were administered 5 times with vehicle (5% DMSO) or baicalein under the same protocol to determine whether baicalein could affect DA metabolism in intact mice, striatal DA levels

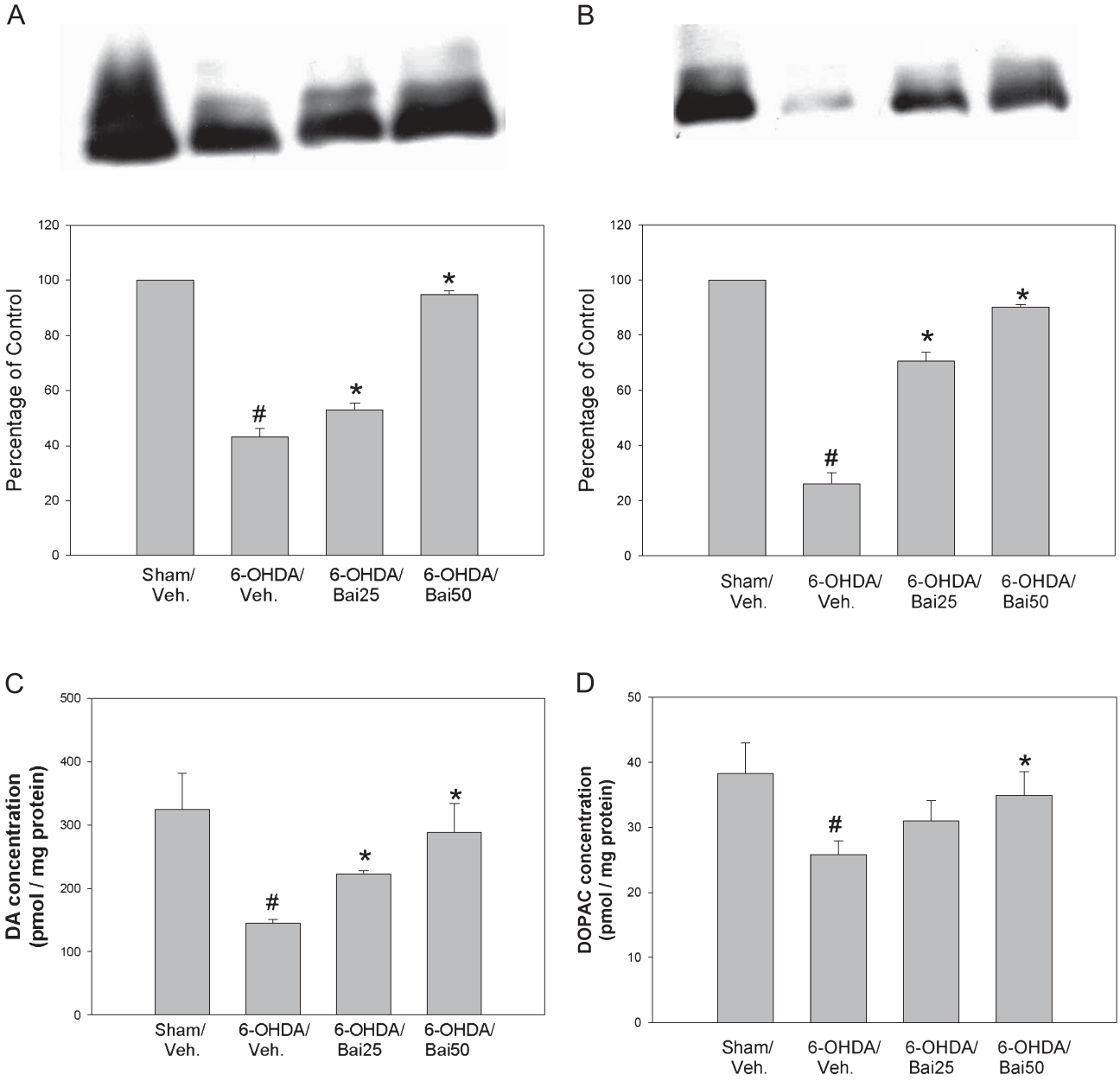

Fig. 2. Effects of baicalein on tyrosine hydroxylase (TH) contents in the striatum and SN and the levels of dopamine (DA) and DOPAC in the striatum with 6-hydroxydopamine injection. Seven days after 6-OHDA injection, TH contents were measured by Western blotting, and DA and DOPAC levels were measured by HPLC. Six to seven mice were used for each experimental group. Data were expressed as the mean ± S.E.M. Representative TH Western blot (top) and quantitative analysis (bottom) in the striatum (A) and SN (B). C: Striatal DA level, D: DOPAC level. *P<0.05: 6-OHDA/vehicle *vs* 6-OHDA/baicalein, $^{\#}P$<0.05: Sham/vehicle *vs* 6-OHDA/vehicle.

were not significantly changed in intact mice, indicating that baicalein itself does not influence the level of DA and its metabolites in this experimental condition (data not shown). Levels of the DA metabolite DOPAC were also decreased by 6-OHDA injection (Fig. 2D). However, treatment with a high dose of baicalein showed protective effects against the reduction of DOPAC levels induced by i.c.v. 6-OHDA injection.

To determine the antioxidant effect of baicalein in 6-OHDA-injected mice, MDA formation in the striatum was measured. In 6-OHDA-injected mice, MDA levels in the striatum were increased 2-fold at 3 days after and 1.4-fold at 7 days after when compared to the level of sham-treated mice. However, at 3 days after 6-OHDA injection, baicalein treatment at 25 and 50 mg/kg reduced MDA levels to 141.6 ± 5.9% and 100.2 ± 1.4%,

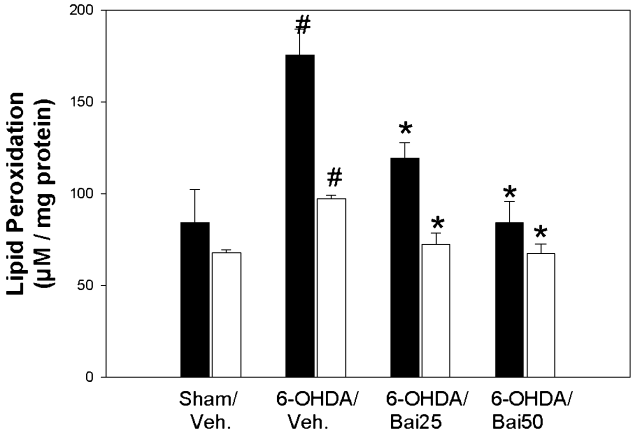

Fig. 3. The effects of baicalein treatment on striatal malondialdehyde (MDA) levels in 6-hydroxydopamine injection. Eight to ten mice were used for each experimental group. Data were expressed as the mean ± S.E.M. **P*<0.05: 6-OHDA/vehicle *vs* 6-OHDA/baicalein, #*P*<0.05: Sham/vehicle *vs* 6-OHDA/vehicle. Closed columns: 3 days after i.c.v. injection; open columns: 7 days after i.c.v. injection.

respectively, the level in sham-treated mice. Furthermore, at 7 days after 6-OHDA injection, the treatment of both doses of baicalein almost completely reduced the MDA levels to that of sham-treated mice (Fig. 3).

In this study, we demonstrated that the baicalein treatment prevented the 6-OHDA-induced dopaminergic dysfunction, as confirmed by the improvement from the 6-OHDA-induced reduction of latency time to fall off the rotarod, decreases in contents of TH and DA and its metabolites as well as by elevation of MDA in the striatum. Recently, there have been some reports that drug-free behavioral tests such as the rotarod test have been used for the parkinsonian animal model, especially for evaluating bilateral dopaminergic dysfunction (12, 13). In this study, we used the accelerating rotarod test to evaluate the 6-OHDA-induced behavioral change because i.c.v. injection of 6-OHDA causes bilateral dopaminergic degeneration. The result of rotarod test suggest that baicalein treatment prevents the dopaminergic dysfunction induced by 6-OHDA injection, even though the exact mechanism underlying the impairment of rotarod performance in 6-OHDA-injected animals require further characterization because the rotarod test is not specific for evaluating the impairment induced by loss of DA neurotransmitter in brain.

A number of previous studies showed that intraventricular administration of 6-OHDA appeared to produce widespread loss of DA terminals in the striatum with a marked reduction in both DA and DOPAC content and TH activity (14). These studies are consistent with our results of the decrease of DA and DOPAC levels in the striatum and TH contents in the striatum and SN by i.c.v injection of 6-OHDA. However, baicalein prevents the 6-OHDA-induced reduction of DA and TH contents in the striatum and SN.

Ogawa et al. (15) reported that a single i.c.v. injection of 6-OHDA in mice resulted in a biphasic increase in lipid peroxidation as assayed by the level of thiobarbituric-acid-reacting substances. In Ogawa's report, lipid peroxidation was initially increased (within 1 – 3 days) and then normalized within a fairly short period of time (within 7 days), followed by a gradual increase again. However, our results showed that striatal lipid peroxidation was significantly increased at 3 days after 6-OHDA injection and was less elevated at 7 days after 6-OHDA injection. However, 6-OHDA-induced increases in the lipid peroxidation product MDA returned to the control levels by baicalein treatment. These results indicated that baicalein has an antioxidative effect against 6-OHDA toxicity.

In conclusion, the present study showed that baicalein effectively prevented the 6-OHDA-induced dopaminergic dysfunction. Moreover, these results suggest that baicalein can be useful agent for the prevention or treatment of neurodegenerative diseases such as PD.

Acknowledgment

This research was supported by a grant (PF002102-00) from the Plant Diversity Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of the Korean government and Brain Korea 21.

References

1 Esposito E, Rotilio D, Di Matteo V, Di Giulio C, Cacchio M, Algeri S. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. Neurobiol Aging. 2002;237:19–35.
2 Beal MF. Mitochondria, free radicals, and neurodegeneration. Curr Opin Neurobiol. 1996;6:661–666.
3 Bowling AC, Beal MF. Bioenergetic and oxidative stress in neurodegenerative diseases. Life Sci. 1995;56:1151–1171.
4 Hou JG, Cohen G, Mytilineou C. Basic fibroblast growth factor stimulation of glial cells protects dopamine neurons from 6-hydroxydopamine toxicity: involvement of the glutathione system. J Neurochem. 1997;69:76–83.
5 Datla KP, Christidou M, Widmer WW, Rooprai HK, Dexter DT. Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease. Neuroreport. 2001;12:3871–3875.
6 Kim MS, Lee JI, Lee WY, Kim SE. Neuroprotective effect of Ginkgo biloba L. extract in a rat model of Parkinson's disease. Phytother Res. 2004;18:663–666.
7 Den-en Shieh, Li-the Liu, Chun-Ching Lin. Antioxidant and free

radical scavenging effects of baicalein, baicalin and wogonin. Anticancer Res. 2000;20:2861–2866.
8 Morel I, Lescoat G, Cogrel P, Sergent O, Pasdeloup N, Brissot P, et al. Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem Pharmacol. 1993;45:13–19.
9 Kuo SM, Leavitt PS, Lin CP. Dietary flavonoids interact with trace metals and affect metallothionein level in human intestinal cells. Biol Trace Element Res. 1998;62:135–153.
10 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.
11 Ara J, Przedborski S, Naini AB, Jackson-Lewis V, Trifiletti RR, Horwitz J, et al. Inactivation of tyrosine hydroxylase by nitration following exposure to peroxynitrite and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Proc Natl Acad Sci USA. 1998;95:7659–7663.
12 Rozas G, Labandeira Garcia JL. Drug-free evaluation of rat models of parkinsonism and nigral grafts using a new automated rotarod test. Brain Res. 1997;749:188–199.
13 Spooren WP, Gasparini F, Bergmann R, Kuhn R. Effects of the prototypical mGlu(5) receptor antagonist 2-methyl-6-(phenyl-ethynyl)-pyridine on rotarod, locomotor activity and rotational responses in unilateral 6-OHDA-lesioned rats. Eur J Pharmacol. 2000;406:403–410.
14 Onn SP, Berger TW, Stricker EM, Zigmond MJ. Effects of intraventricular 6-hydroxydopamine on the dopaminergic innervation of striatum: histochemical and neurochemical analysis. Brain Res. 1986;376:8–19.
15 Ogawa N, Asanuma M, Kondo Y, Hirata H, Nishibayashi S, Mori A. Changes in lipid peroxidation, Cu/Zn-superoxide dismutase and its mRNA following an intracerebroventricular injection of 6-hydroxydopamine in mice. Brain Res. 1994; 646:337–340.