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Baicalein Protects 6-OHDA-induced Neuronal Damage by Suppressing Oxidative Stress

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The protective effects of baicalein, one of the flavonoids in Scutellaria baicalensis Georgi, were evaluated against 6-hydroxydopamine (6-OHDA)-induced neuronal damage in mice and cultured human neuroblastoma cells. Nigrostriatal damage was induced by stereotaxically injecting 6-OHDA into the right striatum. Baicalein was administered intraperitoneally 30 min before and 90 min after lesion induction. Animals received a further daily injection of baicalein for 3 consecutive days. Two weeks after 6-OHDA injection, contralateral rotational asymmetry was observed by apomorphine challenge in lesioned mice. Tyrosine hydroxylase (TH) immunohistochemistry revealed a significant loss of terminals in lesioned striatum and the reduction of the numbers of TH-positive cell in the ipsilateral substantia nigra (SN). In addition, the levels of dopamine (DA) and DA metabolites were reduced and lipid peroxidation was increased in lesioned striatum. However, baicalein treatment reduced apomorphine-induced rotational behavior in 6-OHDA-lesioned mice, and increased TH immunoreactivity in the striatum and SN, and DA levels in lesioned striatum. Lipid peroxidation induced by 6-OHDA was also inhibited by baicalein treatment. Furthermore, when SH-SY5Y human neuroblastoma cells were treated with baicalein, 6-OHDA-induced cytotoxicity and reactive oxygen species (ROS) production were significantly reduced. These results indicate that baicalein effectively protects 6-OHDA-induced neuronal damage through antioxidant action.

Key Words: 6-OHDA, Baicalein, Tyrosine hydroxylase, Dopamine, Oxidative stress, Antioxidant

INTRODUCTION

Flavonoids are a group of low-molecular-weight polyphenolic compounds of plant origin. They exhibit a variety of biological activities, such as antioxidants, anti-inflammatory, antibacterial, antiviral, antitumor effects (Esposito et al, 2002). During the past decade, flavonoids have been reported to be ideal candidates for reducing oxidative stress, since they possess free radical scavenging (Rice Evans et al, 1995; Saija et al, 1995) and metal ion chelating properties (Morel et al, 1993), and increase the expressions of certain antioxidant proteins (Cai & Wei, 1996; Sudheesh et al, 1999). Moreover, many flavonoids such as quercetin, luteolin, and catechins are better antioxidants than antioxidant nutrients like vitamin C, vitamin E, and β-carotene (van Acker et al, 1996; van Acker et al, 1998; Pietta, 2000; Esposito et al, 2002). Baicalein, the major active component of *Scutellaria baicalensis*, has also been reported to exhibit a neuroprotective effect via its potent antioxidant effect (Wakabayashi, 1999; Lee et al, 2003; Li et al, 2005), and to promote neuronal survival in primary cultured central neurons exposed to glutamate- and glucose deprivation-induced neuronal death (Lee et al, 2003). Baicalein has also been demonstrated to have neuroprotective effects *in vivo*, e.g., it reduced ischemic reperfusion brain injury and neutrophil infiltration in rats (Hwang et al, 2002).

The central nervous system (CNS) shows an exceptionally high degree of vulnerability to reactive oxygen species. Considerable evidence suggests that free radical formation and oxidative stress might play an important role in the pathogenesis of Parkinson's disease (PD) (Olanow, 1993; Russel & Reiter, 1998). In addition, reactive oxygen radicals are involved in the toxicity of 6-hydroxydopamine (6-OHDA)-induced nigrostriatal lesions that is used as an experimental model of Parkinson's disease (Gerlach & Riederer, 1996).

The injection of 6-OHDA to lateral ventricle of rats or mice produces the widespread loss of DA terminals through striatum. However, partial recovery of the dopaminergic innervation of striatum occurs within several months after 6-OHDA treatment (Onn et al., 1986; Luthman et al., 1994; Bensadoun et al., 1998). Previously, we also reported that baicalein prevents dopaminergic dysfunction induced by intracerebroventricular (i.c.v.) injection of 6-OHDA in mice by maintaining striatal dopamine (DA) level and nigral TH expression (Im et al, 2005). However, it is not clear whether

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ABBREVIATIONS: 6-OHDA, 6-hydroxydopamine; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; PD, Parkinson disease; ROS, reactive oxygen species; SN, substantia nigra; TH, tyrosine hydroxylase.

baicalein can protect the 6-OHDA-induced dopaminergic neuronal death in mice, because i.c.v. injection of 6-OHDA usually decreases dopaminergic neuronal function rather than inducing neuronal death.

To elucidate the protective effects of baicalein on dopaminergic neuronal loss, we used an animal model of PD produced by the stereotaxic injection of 6-OHDA into mice striatum (Rebekah et al, 2005; Ruxandra et al, 2005). Behavioral, biochemical and immunohistochemical changes in striatum and SN were determined following baicalein treatment. We also investigated the mechanism by which baicalein protects neuroblastoma cells by modulating ROS production.

METHODS

Animals and intrastriatal injection of 6-OHDA

Mice were housed in a temperature-controlled room under a 12-h light/12-dark cycle with free access to food and water. Animals were treated in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised in 1996). Desipramine (25 mg/kg) was injected intraperitoneally (i.p.) to male ICR mice (26~28 g, Daehan Biolink co., Ltd., Korea) to block norepinephrine reuptake 1 h before 6-OHDA injection. Animals were anesthetized with equithesin (0.6 mg/ml, 5 ml/kg, i.p.) and injected 10 μg of 6- OHDA (4 μg/μl containing 0.2 mg/ml L-ascorbic acid, Sigma, St. Louis, MO, USA). 6-OHDA was stereotaxically injected into the right striatum (AP 0.8 mm, ML 2.0 mm, DV 3.3 mm from bregma and dura) at 0.5 μl/min using a 26-gauge Hamilton syringe. Baicalein (25 or 50 mg/kg, i.p., Aldrich, St. Louis, MO) dissolved in 5% DMSO was administered 30 min before and 90 min after 6-OHDA injection. Animals received daily doses of baicalein for 3 consecutive days following 6-OHDA injection. In addition, sham (L-ascorbic acid)-injected mice received baicalein or 5% DMSO (instead of baicalein), and 6-OHDA-injected mice received 5% DMSO.

Behavioral studies

Drug-induced asymmetric rotational behaviors were tested using apomorphine (0.5 mg/kg, s.c.) 2 weeks after lesioning. Mice were placed in individual plastic bowls with a diameter of 20 cm, and rotational behavior was assessed using automated Rotometer (Ruxandra et al, 2005). Mice were acclimatized with their environment for 10 min before turns. And the number of net rotations (contralateral- ipsilateral) of animals were recorded for 60 min.

The striatal levels of DA and DA metabolites

Two weeks after 6-OHDA injection, striatal tissues were collected immediately after animals had been sacrificed, and then frozen on dry-ice. Tissues were homogenized in homogenizing buffer (0.1 M perchloric acid, 0.1 mM EDTA), and dihydroxybenzylamine (DHBA) was added as an internal standard. To measure the levels of DA and its metabolites, homogenized samples were centrifuged at 12,000 g for 10 min. The supernatant obtained was then filtered (0.2 μm, Millipore), and 10 μl aliquot was injected onto a mBondapak C18 (3.9×300 mm column, Waters, Milford, MA). A HPLC unit (GILSON 307) equipped with an electrochemical detector (TOA ICA-5212 system, TOA Electronics Ltd.) was used to determine the striatal levels of DA and DOPAC, as described by Ara et al (1998). The mobile phase consisted of 70 mM monobasic sodium phosphate, 1 mM sodium octansulfonic acid, 0.1 μM EDTA and 5% acetonitrile (pH 3.2 with perchloric acid). The potential of the electrochemical detector was set at 700 mV.

Tissue preparation and histological procedure

After behavioral testing, animals were deeply anaesthetized with equithesin (3 ml/kg, i.p.), transcardially perfused with normal saline supplemented with heparin (143 USP units), and fixed in 4% paraformaldehyde dissolved in 0.1M phosphate buffer (PB). Brains were removed, post-fixed for 1 h, washed in 0.1 M PB, and equilibrated in 30% sucrose overnight. Six series of free-floating 40 μm thick coronal sections were prepared for immunohistochemistry and then processed for immunostaining using mouse monoclonal anti-tyrosine hydroxylase (TH) (Chemicon, Temecula, CA, USA). Free-floating tissue sections were rinsed twice in 0.1 M phosphate-buffered saline (PBS) for 10 min, and incubated in 0.2% Triton X-100 dissolved in 0.1 M PBS and 1% bovine serum albumin (PBS/0.5% BSA). Sections were incubated at room temperature with TH antibodies (1 : 500) overnight, and rinsed in PBS/0.5% BSA the following day. The appropriate biotinylated secondary antibodies (1 : 200) were visualized by incubating tissue in 0.05% diaminobenzidine HCl and 0.003% hydrogen peroxide. Tissues were rinsed in 0.1 M PB, mounted on a gelatin-coated slide, air-dried, dehydrated, coverslipped with permount and analyzed under a bright-field microscope. Cresyl Violet staining was performed to determine the site of lesion.

Quantitative analysis

Briefly, TH-positive neurons of the substantia nigra pars compacta (SNC) were counted manually (light microscopy; ×400) using a superimposed grid to facilitate the procedure. At least two sections, which were representative of each of four Paxinos?Watson planes (4.2, 3.8, 3.2, 2.97; Interaural), were examined by scanning the entire extent on each side. Counting was done blind to the treatment received. The number of SNC neurons was expressed as the average of counts obtained from the representative sections for an averaged area of 288 μm. The area of the SNC was drawn by a camera lucida (Wild, Switzerland; × 10) and measured by a mechanical planimeter. A similar method was used for counting WGA-HRP-labeled neurons.

Lipid peroxidation assay

Two weeks after 6-OHDA administration, striatal tissues were collected immediately after animals had been sacrificed, and then frozen on dry-ice and stored at −70°C until required. Tissue was weighed and homogenized in homogenizing buffer (10 mM K_2HPO_4, KH_2PO_4, 30 mM KCl, 1 mM EDTA, pH 7.4). To measure malondialdehyde (MDA) levels, 300 μl of homogenized tissue was mixed with a cocktail solution containing 8.1% SDS, 20% acetic acid, and 0.67% thiobarbituric acid. This solution was shaken vigorously, boiled for 1 h, cooled with tap water, and centrifuged at 1,000 g for 10 min at room temperature. Lipid peroxidation levels were used to calculate MDA levels by measuring

absorbance at 532 nm. Concentrations are expressed as nmoles MDA/mg protein.

The Cytotoxic effect of 6-OHDA on neuroblastoma cells

SH-SY5Y human neuroblastoma cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum, containing 100 U/ml penicillin and 100 μg/ml streptomycin, under a 5% CO_2 humidified atmosphere at 37°C. Approximately 5×10^3 cells per well were seeded into 96 well plates and maintained with DMEM, supplemented with 1% FBS, for 4 h after cells had attached to well surfaces. Cells were pre-incubated with 5 or 10 μM baicalein for 2 h before being exposed to 20 μM 6-OHDA. After 24 h of incubation, media were replenished and 10 μl of 3-(4,5-dimethyl-2-thiazoyl)-2, 5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) was added to each well. Incubation was continued for 2 h at 37°C, and the formazan crystals formed were solubilized with DMSO and quantified by measuring absorbance at 570 nm using an ELISA reader.

Reactive oxygen species (ROS) measurements

Hydrogen peroxide or intracellular oxidant-generation induced by 6-OHDA was measured by incubation of samples with the fluorescent probe 2', 7'-dichlorofluorescein diacetate (DCF-DA; Sigma, St. Louis, MO, USA). Cells were grown in 35 mm dishes (4×10^5/dish) and pre-incubated with 10 μM baicalein for 2 h before being exposed to 20 μM 6-OHDA. DCF-DA (5 μM) was then loaded for 15 min, 90 or 180 min after being exposed to 6-OHDA. SH-SY5Y cells were washed twice with phosphate-buffered saline (PBS). Cells were lysed and aliquoted into 96 well plates (20 μg/well). DCF fluorescein intensity was measured by fluorometry (excitation 485 nm, emission 530 nm; TECAN, GENios, Maennedort, Switzerland).

Statistical analyses

All data are presented as means ± S.E.M. Statistical comparison between different treatments were performed using one-way analysis of variance (ANOVA) with Duncan's multiple test. Significance was accepted for p values of < 0.05.

RESULTS

Effect of baicalein on apomorphine-induced rotation induced by striatal 6-OHDA injection

Apomorphine-induced rotation was neither increased nor different in either direction in sham-injected animals (Fig. 1). In contrast, mice exhibited contralateral rotational behavior following apomorphine challenge in 2 weeks after the unilateral administration of 6-OHDA into the striatum. Significant increases in the number of apomorphine-induced rotations were observed compared with sham-treated mice (312.2 ± 13.3 vs. 23.8 ± 2.8 turns/h). Attenuation of asymmetric motor behavior was observed in experimental animals treated with baicalein; i.e., 214.7 ± 14.6 turns/h for 25 mg/kg and 129.1 ± 2.7 turns/h for 50 mg/kg, respectively (Fig. 1).

Effects of baicalein on DA and DA metabolite levels induced by striatal 6-OHDA injection

The levels of striatal DA and its metabolites were determined after 6-OHDA injection. DA levels in sham-injected with vehicle- or baicalein-treated mice were 842.7 ± 32.0 pmol/mg protein or 905.2 ± 51.0 pmol/mg protein, respectively. In contrast, the mean DA level in the ipsilateral sides

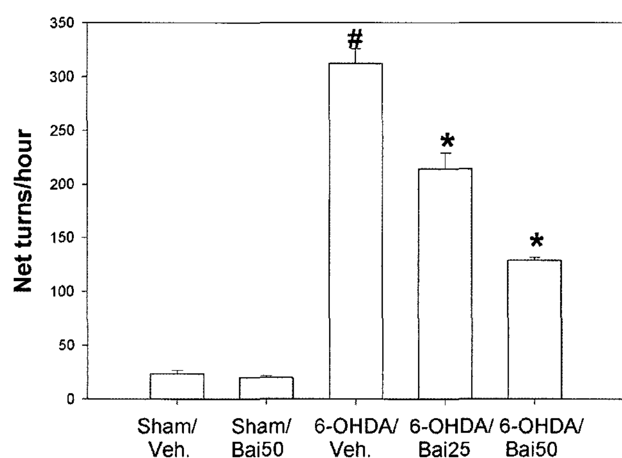

Fig. 1. Effects of baicalein treatment on apomorphine (0.5 mg/kg, s.c.)-induced rotational behavior in 6-OHDA-lesioned mice. Two weeks after 6-OHDA injection, the number of net rotation contralateral to the lesion side was increased. However, baicalein treatment (25, 50 mg/kg) restored the decrease of number of net rotation. 8~10 animals were assigned to each experimental group. All data are represented as means ± SEM. *p < 0.05: 6-OHDA + vehicle vs. 6-OHDA + baicalein; #p < 0.05: sham + vehicle vs. 6-OHDA + vehicle.

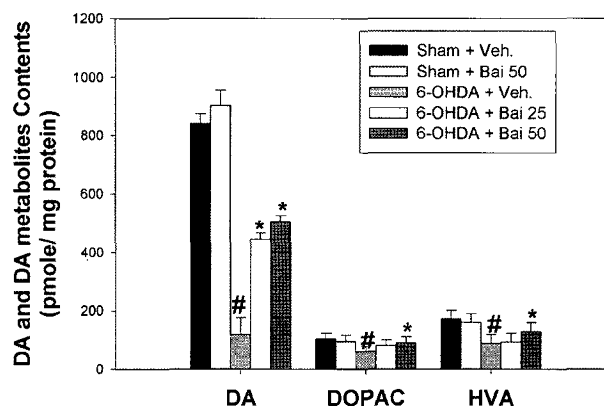

Fig. 2. Effects of baicalein treatment on striatal DA (A) and DA metabolites (B, C) levels in 6-OHDA-lesioned mice. Two weeks after 6-OHDA injection, DA (A), DOPAC (B), and HVA (C) levels were significantly reduced by 6-OHDA. However, baicalein treatment increased striatal DA levels in a dose-dependent manner. 8~10 animals were assigned to each experimental group. All data are represented as means ± SEM. *p < 0.05: 6-OHDA + vehicle vs. 6-OHDA + baicalein; #p < 0.05: sham + vehicle vs. 6-OHDA + vehicle.

of 6-OHDA treated mice was significantly reduced to 118.2±58.1 pmol/mg protein. However, baicalein treatment dose-dependently increased mean striatal DA level in the ipsilateral side to 444.4±22.3 pmol/mg protein (25 mg/kg baicalein treatment) and 505.2±19.8 pmol/mg protein (50 mg/kg baicalein treatment). The levels of the dopamine metabolites DOPAC and HVA were also reduced by 6-OHDA administration, and baicalein treatment showed a similar preventive effect. These results show that baicalein prevents 6-OHDA-induced striatal DA loss (Fig. 2).

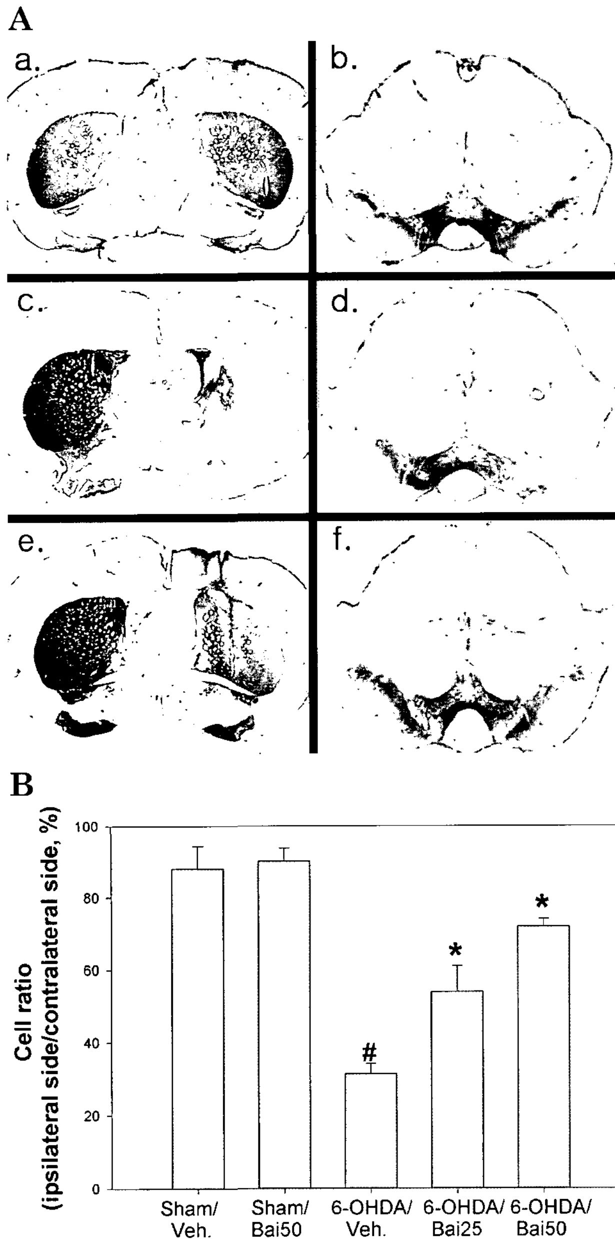

Fig. 3. Effect of baicalein treatment on TH immunohistochemical staining in the SN (b, d, f) and striatum (a, c, e) in 6-OHDA-lesioned mice. (A). Notice the loss of cell bodies in SN (d) and the absence of terminals in the dorsolateral striatum (c) ipsilateral to the lesioned side, when compared to the control (a, b). Baicalein treatment (50 mg/kg; e, f) appeared to improve the number of neurons in the SN and the survival of terminals in the dorsolateral striatum (B). Quantification of TH immunopositive neurons in the SN. Notice the significant dose-dependent increases in the numbers of TH immunostained neurons in the SN following baicalein treatment (25, 50 mg/kg). 8~10 animals were assigned to each experimental group. All data are represented as means±SEM. *$p<0.05$: 6-OHDA+vehicle vs. 6-OHDA+baicalein; #$p<0.05$: sham+vehicle vs. 6-OHDA+vehicle.

Effect of baicalein on reduced TH-immunoreactivity induced by 6-OHDA administration

In order to examine whether the neuroprotection exerted by baicalein, as indicated by striatal DA and TH levels, was a consequence of neuronal survival or a compensatory event, we used an immunohistochemical approach. Histochemical analysis using mouse anti-TH antibody revealed extensive denervation of immunopositive fibers and cell bodies within the ipsilateral striatum and SN after 6-OHDA administration, respectively (Fig 3A-c, d), and baicalein significantly restored the reduced TH immunoreactivity induced by 6-OHDA in the striata and SNs (Fig. 3A-e, f). The levels of TH+ neurons in sham-operated side showed a mean of 259.8±2.4, and those of 6-OHDA injected side were 81.3±3.1 cells (Fig. 3B). However, baicalein treatment (25 mg/kg) partially blocked this killing of dopaminergic cells by 6-OHDA, with a mean of 188.0±9.0 cells. However, TH+ cells in ipsilateral SN of the baicalein (50 mg/kg) treated group showed a mean of 212.2±4.0 cells, which was similar to that of the sham group. In addition, baicalein alone (without 6-OHDA) showed no evidence of altering TH immunoreactivity. These results indicate that baicalein treatment prevented 6-OHDA-induced dopaminergic neuronal loss.

Effects of baicalein on the generation of MDA induced by 6-OHDA administration

Next, we examined the antioxidant properties of baicalein. Two weeks after administering 6-OHDA, the lipid peroxidation product (MDA) level of lesioned striatum was

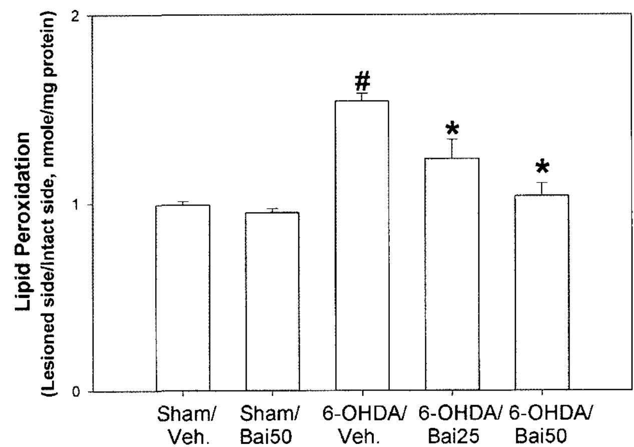

Fig. 4. Effects of baicalein treatment on striatal MDA levels in 6-OHDA-lesioned mice. Notice the return of MDA levels to normal following baicalein treatment. 8~10 animals were assigned to each experimental group. All data are means±SEM. *$p<0.05$: 6-OHDA+vehicle vs. 6-OHDA+baicalein; #$p<0.05$: sham+vehicle vs. 6-OHDA+vehicle.

increased to 1.54±0.04 nmole/mg protein compared to those of contralateral sides (1.01±0.15 nmole/mg protein). In contrast, in sham-treated mice, no difference was observed in MDA levels between contralateral and ipsilateral sides of the striatum (0.99±0.02 and 0.97±0.12 nmole/mg protein). However, baicalein treatment at 25 or 50 mg/kg reduced mean MDA levels in ipsilateral sides to 1.23±0.98 and 1.03±0.07 nmole/mg protein, respectively (Fig. 4). These results indicate that baicalein treatment inhibited 6-OHDA-induced lipid peroxidation in the striatum.

Effects of baicalein on the cytotoxicity of 6-OHDA and ROS generation by 6-OHDA in SH-SY5Y neuroblastoma cells

To evaluate the protective effects of baicalein against 6-OHDA-induced cytotoxicity, SH-SY5Y neuroblastoma cells were treated with 20 μM 6-OHDA for 24 h in the presence or absence of baicalein. The cellular damage induced by 6-OHDA was assessed by determining cell viability using the MTT test (Fig. 5A). Mean MTT reduction levels in 20 μM 6-OHDA treated cells for 24 h reduced to 45.0±2.2% of the control level after incubating with 6-OHDA. Baicalein pretreatment (5 or 10 μM) significantly protected SH-SY5Y cells from this 6-OHDA-induced cytotoxicity, and MTT was reduced to 80.1±1.9% and 82.0±0.9%.

ROS generation was measured by using the cell permeable dye DCFH-DA, which becomes highly fluorescent when oxidized to DCF. Thus, DCFH-DA was loaded into cells after 6-OHDA treatment, and changes in fluorescence at different time points were measured using a fluorometer. Fluorescence increases were detected as early as 90 min after treatment with 6-OHDA, and DCF fluorescence was significantly elevated to 245% of the control level after 3 h, but baicalein treatment (10 μM) reduced this to 170% of the control level (Fig. 5B).

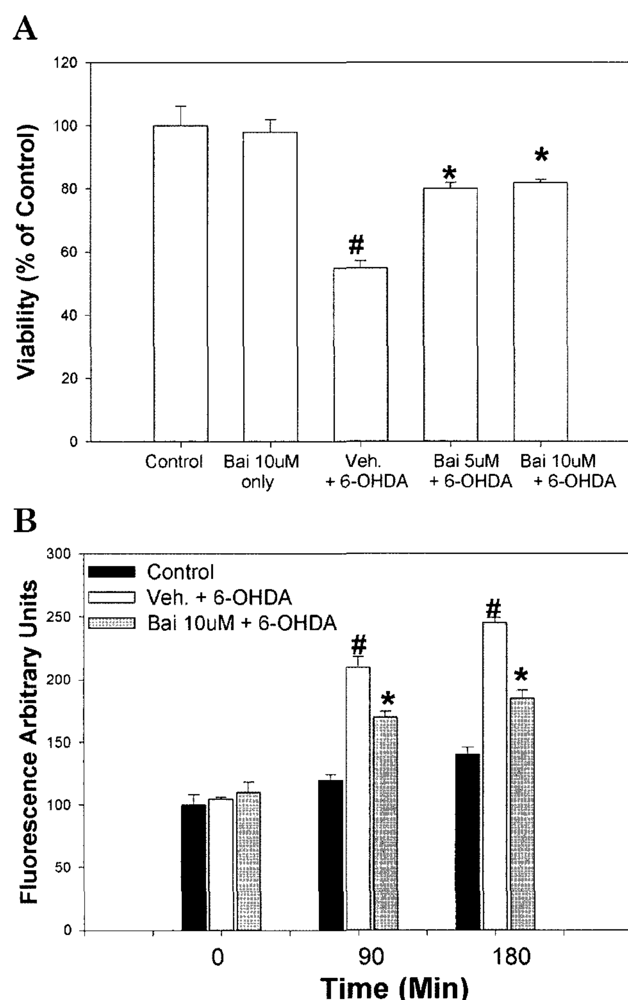

Fig. 5. Effect of baicalein on MTT reduction and ROS generation after treatment with 6-OHDA (A). Cells were pretreated for 2 h with baicalein (at 5 or 10 μM), and this significantly prevented the MTT reduction induced by 6-OHDA (B). Cells were exposed to 6-OHDA (20 μM) for 90 or 180 min. Increased DCF fluorescence by 6-OHDA treatment was blocked by baicalein (10 μM). All data are means±SEM. *$p<0.05$: 6-OHDA only vs. 6-OHDA+baicalein; #$p<0.05$: control vs. 6-OHDA only.

DISCUSSION

The present results demonstrated that baicalein can protect dopaminergic neurons from 6-OHDA challenge, confirmed by restoration of a number of TH-positive neurons in SNpc, levels of striatal DA and its metabolite, and behavior in 6-OHDA-lesioned mice. Moreover, lipid peroxidation in the lesioned striatum and ROS generation in a neuroblastoma cell line were reduced by baicalein treatment.

A number of studies demonstrate that the intrastriatal administration of 6-OHDA in rat markedly decreases TH-immunoreactive nerve terminals in the striatum as well as cell bodies in the SN ipsilateral to 6-OHDA-lesioned side (Rebekah et al, 2005; Ruxandra et al, 2005). These studies are comparable to our results of the absence of TH-immunoreactive nerve terminals in the dorsolateral striatum and significant loss of TH-positive neurons in the ipsilateral SN in mouse after 6-OHDA challenge. The morphological deficit caused by 6-OHDA in rats and mice causes characteristic motor dysfunctions that may be demonstrated by treating with DA receptor agonists, such as apomorphine, which result in a rotation in a direction contralateral to the lesioned side (Iancu et al, 2005; Ruxandra et al, 2005). The appearance of apomorphine-induced rotation involves DA receptor supersensitivity due to a loss of striatal TH terminals, resulting in a significant reduction of DA and DA metabolites levels in the 6-OHDA-lesioned striatum (Carmen et al, 1991; Fisher et al, 1991). In the present study, the finding that baicalein treatment ameliorated the drug-induced asymmetric rotations concomitant with a partial restoration of DA levels suggests that baicalein may have a protective effect on the denervated striatum that is associated with a subsequent reduction of receptor supersensitivity. The survival of striatal TH terminals and their cell bodies in the SN following baicalein treatment may contribute to a recovery of motor dysfunction in the 6-OHDA-lesioned striatum.

The present study showed that 6-OHDA-induced increase of MDA returned to control levels by baicalein treatment. Additionally, ROS production induced by 6-OHDA, confirmed *in vitro* by a DCF method, was reduced by baicalein treatment. Earlier studies with 6-OHDA indicated that this neurotoxin is highly reactive, and that it is readily autooxidized and oxidatively deaminated by monoamine oxidase

to produce hydrogen peroxide and ROS (Cohen & Heikkila, 1974). 6-OHDA has also been suggested to exert its neurodegenerative action via oxidative stress (Glinka et al, 1996; Glinka et al, 1997): Oxidative stress causes ROS generation, followed by brain membrane lipid peroxidation. Thus, oxidative damage caused by 6-OHDA may be prevented by antioxidant effect. These earlier reports are in agreement with our present result that the level of lipid peroxidation product, MDA (an index of oxidative stress), was increased in the lesioned striatum by 6-OHDA. These results support the notion that baicalein acts as an antioxidant by scavenging free radicals.

Recent studies have shown that flavonoids have many beneficial pharmacological effects including anti-inflammatory, anticancer, and antioxidant properties. Flavonoids are known to scavenge free radicals (Rice et al, 1995; Pietta, 2000) and chelate metal ions (Li et al, 2005) or increase the expression of antioxidant proteins (Cai & Wei, 1996). Furthermore, there are several reports to show that baicalein attenuates the cellular injury induced by hydrogen peroxide (Cohen & Heikkila, 1974) and β-amyloid (25~35) induced toxicity (Lebeau et al, 2001) by it's ROS scavenging/antioxidant action, that flavonoids (EGCG, catechin) or flavonoid containing compound such as EGb761 protect the neurotoxicity induced by MPTP, MPP or 6-OHDA *in vitro*, and that flavonoids have neuroprotective effects in MPTP-induced animal model of parkinsonian. Recently, the citrus flavonoid tangeretin (Datla et al, 2001) and Ginkgo biloba L. extract (Kim et al, 2004) have been shown to protect the dopaminergic neuronal damage in a rat model of Parkinson's disease, and Levites and his colleagues studied neuroprotective property of green tea extract and (-)-epigallocatechin-3-gallate in MPTP mice model (Levites et al, 2001). Nevertheless, the protective effect of flavonoids, including baicalein, in 6-OHDA-induced animal model has not been extensively studied.

Taken together, our present results indicate that baicalein effectively protects the 6-OHDA-induced dopaminergic neuronal damage by acting as an antioxidant. Behavioral, histochemical and biochemical effects of baicalein in the animal PD model may provide useful therapeutic strategies for the treatment of human PD.

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