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

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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 baicalein 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 substantia 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

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 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).
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 micro-liters of the samples was injected onto a µ-Bondapak C18 (3.9 × 300 mm; 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 K2HPO4, KH2PO4, 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.

TH contents in the striatum and SN were determined by Western blotting. Striatal TH content was markedly increased 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...
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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%,
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.

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![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.](image-url)