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Baicalein attenuates 6-hydroxydopamine-induced neurotoxicity in SH-SY5Y cells

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Abstract

It has been suggested that baicalein, a flavonoid obtained from the *Scutellaria* root, exerts a protective role on neurons against several neuronal insults. However, the protective mechanisms underlying this protective effect remain largely unknown. Our results indicate that baicalein protects SH-SY5Y cells, a dopaminergic neuronal cell line, from 6-hydroxydopamine (6-OHDA)-induced damage by the attenuation of reactive oxygen species (ROS). In order to determine the effects of baicalein on mitochondrial events, mitochondrial membrane potentials ($\Delta\Psi_m$) and caspase cascades downstream of mitochondria were assessed. Baicalein inhibited the collapse of $\Delta\Psi_m$, suggesting that baicalein reduces the mitochondrial dysfunction associated with 6-OHDA treatment. Baicalein also inhibited caspase-9 and caspase-3 activation, which can be triggered by mitochondrial malfunctions. Furthermore, baicalein induced a significant reduction in the level of phospho-JNK, which is known as an apoptotic mediator in 6-OHDA-induced neuronal cell death. Our results indicate that baicalein protects neurons from the deleterious effects of 6-OHDA via the attenuation of oxidative stress, mitochondrial dysfunction, caspase activity, and JNK activation.

Keywords: Baicalein; 6-Hydroxydopamine (6-OHDA); Mitochondria; Caspase; JNK; Cell death

Introduction

Parkinson's disease (PD) is a typical neurodegenerative disorder, characterized by symptoms including rest tremors, postural instability, gait abnormality, bradykinesia and rigidity. Although genetically susceptible individuals exhibit early onset of PD, in most cases PD occurs sporadically with age (Alonso et al., 1986). PD is mainly characterized by the selective loss of the dopaminergic neurons in the substantia nigra. The mechanisms by which this neuronal loss occurs remain unclear (Moghal et al., 1994). The presence of apoptotic nigral neurons was demonstrated in post-mortem brains of PD patients (Anglade et al., 1997).

Insight into the potential mechanisms which contribute to PD-associated neurodegeneration has been obtained from animal PD models. One of the neurotoxins which can induce PD in this model is 6-hydroxydopamine (6-OHDA). 6-OHDA is a dopamine

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analog, which readily undergoes non-enzymatic oxidation, resulting in the production of hydrogen peroxide (H_2O_2), superoxide, and the hydroxyl radical, under physiological pH conditions (Cohen and Heikkila, 1974). Intrastriatal 6-OHDA injections result in a Parkinsonian pattern, together with neuronal loss in the substantia nigra of the rat brain (Ungerstedt, 1968). Although used as an exogenous neurotoxin in this model, some evidence suggests that 6-OHDA can be formed in vivo from dopamine. In PD, the neurons within the substantia nigra pars compacta (SNpc) contain an increased amount of iron (Hirsch et al., 1991). In addition, the effects of PD reduce the activities of catalase and glutathione peroxidase in the brain (Ambani et al., 1975; Kish et al., 1985). In the presence of free ferric iron and H_2O_2, the principal product of dopamine oxidation has been reported to be 6-OHDA (Jameson and Linert, 1999). Andrew et al. (1993) reported that the urine of PD patients treated with levodopa contained an increased amount of 6-OHDA. The fact that 6-OHDA is found in the brain and urine samples of PD patients indicates that 6-OHDA may constitute an important endogenous cause of PD pathogenesis (Andrew et al., 1993; Curtius et al., 1974; Jellinger et al., 1995).

Several studies have suggested that baicalein exerts an anti-oxidant effect (Gao et al., 1995; Hanasaki et al., 1994). Also, Hamada et al. (1993) reported that baicalein is capable of scavenging reactive oxygen species (ROS), including superoxide (O^{2-}), H_2O_2, and hydroxyl radicals. Baicalein has also been shown to strongly inhibit iron-dependent lipid peroxidation in microsomes (Gao et al., 1995) and mitochondria (Miyahara et al., 1993). Gao et al. (1998) reported that baicalein prevented ROS-mediated damage of human dermal fibroblast cells better than the iron chelator deferoxamine or hydroxyl radical scavengers (including α-tocopherol and allopurinol).

Dopamine agonists including levodopa and ropinirole have been used in the treatment of PD (Rascol et al., 2000) with significantly greater benefit for patients treated with levodopa than with any other substance. However, patients treated with levodopa develop wearing off, dyskinesias, or on–off motor fluctuation (Parkinson Study Group, 2000). Moreover, levodopa cannot be administered for a long time, as the effectiveness of levodopa may be diminished, due to levodopa resistance (Sandyk et al., 1987). Thus, it is clearly important to search for a fundamental mix of therapeutics for the improvement of PD symptoms. Oxidative stress, one of the major causes of PD, may be a target for treatment.

Therefore, in the present study, we have examined the effects of baicalein on 6-OHDA-induced dopaminergic neuronal cell death in SH-SY5Y cells.

Materials and methods

Cell culture

SH-SY5Y human neuroblastoma cells were cultivated at 37 °C in minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco-BRL) in a 95% humidified air and 5% CO_2 atmosphere. Before treating the cells with 6-OHDA (20 μM) they were cultured in MEM, containing 1% FBS for 2 h to assure the neuronal survival and the morphological integrity of the cells.

Pharmacological treatments

6-OHDA (Sigma Chemical Co.) was dissolved in 2% ascorbic acid and used at a final concentration of 20 μM, which was the dose shown to induce 50% cell death within 24 h. Baicalein was a gift from Dr. Y.S. Kim (SNU, Korea) and dissolved in DMSO. Before adding 6-OHDA, cells were treated with a final concentration of 12.5 μM baicalein for 2 h (DMSO content never exceeded 0.1%). SP600125, a specific inhibitor of JNK/SAPK, was purchased from BIOMOL, and cells were treated with the indicated concentrations 2 h before 6-OHDA treatment.

Cell viability assay (AlamarBlue test)

SH-SY5Y cells were plated on 96-well plates (Nunc, Slangerup, Denmark) at a density of 15,000 cells/well in 100 μl of 10% FBS/MEM and incubated for 24 h. Two hours before 6-OHDA treatment, the medium was replaced with 1% FBS/MEM. At the end of the treatment, 10 μl AlamarBlue (Serotec, Oxford, UK) was aseptically added. The cells were incubated for 3 h, and absorbance was measured at a wavelength of 570 nm with an ELISA Reader (Molecular Devices, Sunnyvale, CA, USA). The cell viability was defined as [(test sample count)−(blank count)/(untreated control count)−(blank count)] × 100 (Shimoke and Chiba, 2001).

Determination of ROS generation

Hydrogen peroxide generation, induced by 6-OHDA, was measured by incubating the SH-SY5Y cells with 10 μM 2′,7′-dichlorofluorescein diacetate (DCF-DA; Sigma) for 15 min. Subsequently, cells were washed twice with phosphate-buffered saline (PBS) and then mounted onto glass slides. Photomicrographs were taken with a fluorescence microscope equipped with UV supply system (Olympus IX70, Tokyo, Japan). For quantification of intracellular ROS levels, cells were loaded with 10 μM DCF-DA for 15 min at 37 °C. DCF