Ⅱ-40

신경독성유발물질인 6-OHDA에 대한 BF-7의 보호 효과

(주)바이오그랜드 BG생명의학연구소 : 이현정*
국립농업과학원 : 여주홍, 이광길
(주)브레인가드 : 박지애, 김정현
중앙대학교 : 정윤희, 김대진, 김성수

The Protective Effect of BF-7 against 6-OHDA-induced Neurotoxicity.

[1]BG Biomedical Research Center, Biogrand Inc., [2]National Academy of Agricultural Science, [3]Brainguard Inc., [4]Department of Anatomy and Cell biology, College of Medicine, Chung-Ang University,
Hyun Jung Lee[1,*], Joo Hong Yeo[2], Kwang Gill Lee[2], Ji Ae Park[3], Jeong Hyun Kim[3], Yoon Hee Chung[4], Dae Jin Kim[4], and Sung Su Kim[4]

Objectives

BF-7, *Bombyx mori* extracts, is known to have cell protective effect against various insults. Parkinson's disease (PD) is a typical neurogenerative disorder. One of the nerutoxin which can induce the PD model is 6-hydroxydopamine (6-OHDA). However, the therapeutic agents which constitute a remedy for PD have not yet been elucidated. In this study, we have examined the effects of BF-7 on 6-OHDA-induced dopaminergic neuronal cell death in the SH-SY5Y cells.

Materials and Methods

Cell culture SH-SY5Y human neuroblastoma cells were cultivated at 37℃ in minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) in 95% humidified air and in 5% CO_2 incubator. The cells were then cultured in MEM, coating 1% FBS for 2 h before treating them with 6-OHDA (20 uM) to assure the neuronal survival and the morphological integrity of the cells.

Cell Viability Assay (Alarmarblue test) SH-SY5Y cells were plated on 96-well plates at a density of 15,000 cells/well, in 100 ul of 10% FBS/MEM and then were incubated for 24 h. 2 h before 6-OHDA treatment, the media was replaced with 1% FBS/MEM. At the end of the treatment, 10 ul of alamarblue agent was aseptically added. The cells were incubated for 3 h and the absorbance of the cells were measured at a wavelength of 570 nm with and ELISA Reader. The background absorbance was measured at 600 nm and was subtracted from the measured absorbance of the cells.

주저자 연락처 (Corresponding author) : 이현정 E-mail : pluto38@gmail.com Tel : 02-826-4825

Determination of ROS generation Cultured cells were treated with 10 μM of DCF-DA (6-carboxy-2',7'-dichloro-dihydrofluorescein diacetate, dicarboxym-ethylester) dissolved in HCSS buffer (20 mM HEPES, 2.3 mM CaCl2, 120 mM NaCl, 10 mM NaOH, 5 mM KCl, 1.6 mM MgCl2, 15mM glucose) and 2% Pluronic F-127 at 37℃ for 30min. DCF fluorescence generated by free radicals in cells were estimated using Olympus IX70 microscope with mercury lamp flouresence (Exc. 485 nm, Emi. 530 nm), captured by CCD camera. Then, image analysis was performed using NIH Image 1.65 program and was measured using Flow cytometry (TECAN, GENios, Maennedort, Switzerland) with excitation at 485 nm and emission at 530 nm.

Analysis of mitochondrial membrane potential The changes in mitochondrial membrane potential ($\Delta\Psi_m$) were estimated using tetramethylrhodamine ethyl ester (TMRE) (Molecular Probes, Leiden, TheNetherlands), a cationic potentiometric dye which accumulates preferentially into energized mitochondria driven by the membrane potential. For estimation of $\Delta\Psi_m$, cells were incubated with 100 nM TMRE for 15 min at 37C and then, TMRE fluorescein intensity was measured with excitation at 549 nm and emission at 574 nm by fluorometer (TECAN, GENios). Intensity of $\Delta\Psi_m$ is expressed as arbitrary units of relative value.

Caspase activity assay In order to assay caspase activity in SK-N-SH cells, 10,106 cells were harvested from each P100 plate and lysed with 1ml lysis buffer (10 mM Tris-Hcl, pH 7.4, 10 mM NaH2PO4, pH 7.4, 130 mM NaCl, 1% Triton X-100, 10 mM NaF). 50 μl of lysate was added into 200 μl of HEPES buffer(40 mM HEPES, pH 7.5, 20% glycerol, 4 mM DTT) with 0.25 mM aVAD-PNA, pan caspase substrate for 1 hr. Caspase activity was measured using ELISA Reader(Molecular Devices) with absorbency at 405nm.

Results and Discussion

6-OHDA-induced dopaminergic neuronal apoptosis is caused principally by ROS generation, JNK/SAPK activation, reduced $\Delta\Psi_m$ and induced caspase activation. These factors are regarded as pathological features in PD, and these features are all sugnificantly attenuated by pre-treatment with BF-7. In other words, our experimental results provides us with evidence that baicalein might be used to cure neurodegenerative disorders, including PD. Moreover, then BF-7-based approach may result in the development of safe and promising therapies in the bear future.