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3-Hydroxykynurenine으로 유도된 신경스트레스에 대한 BF-7 보호 효과

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The Protective Effect of BF-7 against 3-Hydroxykynurenine (3-HK)-induced Neuronal Stress

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Objectives

BF-7, *Bombyx mori* extracts, is known to have protective effect against various stimuli. However, the molecular mechanism of the protective effect has not been well identified.

In this study, we investigated how the BF-7 exerts protective effect against 3-Hydroxykynurenine (3-HK), a general neuronal stress agents, using SK-N-SH human neuroblastoma cells.

Materials and Methods

Cell culture SK-N-SH cells the human neuroblastoma cells, were cultured at 37℃ in minium essential mediun (MEM) supplemented with 10% heat-inactin\vated fetal bovine serume(FBS) in humidified 95% air , 5% CO2 incubator. The cells were transferred to low serum media (1% FBS/MEM) 2 h before the treatment with 3-HK. ***Pharmacological treatments.*** 3-Hydroxykynurenine (3-HK) was obtained from Sigma Chemical Co. (St. Louis MO) and dissolved in DMSO. 250 mM 3-HK was used (a dose to induce cell dealth by 50% within 36 hr.

Cell Viability Assay (alamarBlue test) SK-N-SH cells were plated on 96-well plates (Nunc, Denmark) at a density of 15,000 cells/well, in 100 ml of 10% FBS/RPMI 1640 and incubated for 24 h. Before 2 h 3-HK treatment, the media was replaced with 1% FBS/RPMI 1640. At the end of the treatment, 10ml of alamarBlue (Serotec, wasUK) was aseptically added. The cells were incubated for 3 h and absorbance of the cellsmeasured at a wavelength of 570 nm with an ELISA Reader(Molecular Devices, Sunnyvale, CA).The background absorbance was measured at 600 nm and was subtracted. The cell viability was defined as

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[(test sample count)-(blank count)/(untreated control count)-(blank count)]100 (Shimoke and Chiba, 2001).

Hoechst 33258 staining DNA-bindingfluorochrome bis-benze (Hoechst 33258 dye) was used to observe morphological changes of nuclear chromatin in apoptotic cells. 0.5-3.0 * 10^6 cells were centrifused for 10 min and collected. After being washed with PBS, these cells were fixed for 10 min and followed by fixation with 50 μg/ml paraformaldehyde. Samples were washed with PBS, stained with 16 g/ml of Hoechst dye 33258 for 15 min, washed again with distilled water. Then, 10 u1 aliquots were plated on a slide glass to visualize changes of apoptotic chromatin under a fluorescent microscope.

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.

Analysis of mitohondrial membrane potential ($\triangle \psi_m$) The changes in mitochondrial membrane potential ($\triangle \psi_m$) were estimated using tetramethylrhodamine ethyl ester (TMRE) (Molecular Probes, Eugene, OR, USA) , which is a cationic potentipmetric dye that accumulates preferentially into energized mitochondria driven by the membrane potential. To estimated of $\triangle \psi_m$, cells were incubated with 100 nM TMRE for 15 min at 37℃ and then TMRE fluroscein intensity was measured with excitation at 549 nm and emission at 574 nm using a flurorometer (TECAN. GENios ,Maennedort, Switzeland). Intensity of $\triangle \psi_m$ is expressed as arbitary untis of ralative value. Fluroscence image was observed using an IX70 microscope (Olympus. Tokyo, Japan) equipped with attachments for fluroscnce microscopy.

Results and Discussion

Our result show that incubation of SK-N-SH cells with 3-HK-induced neuronal stress and interestingly, pre-treatment of BF-7 increased cell viabilities against 3-HK. Furthermore, 3-HK-induced cell death was proceeded by increased of Bax expression and mitochondrial dysfunction such as collapse of mitochondrial membrane potential ($\triangle \psi m$), release of cytochrome C from mitochondria into cytoplasm and increase of caspase-9/-3 activities. Also, we identified that BF-7 reduced significantly the Bax expression levels, mitochondrial dysfunction and caspase activities, eventually inhibited the cell death. Our results implicated BF-7 plays a protective role by down-regulating Bax expression level and persisting mitochondrial function in 3-HK-induced neuronal cell death.