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# 도파민성 신경세포사멸을 유도하는 1-metyl-4-phenylpyridinium ion ($MPP^+$)의 작용에 대한 키누레닌산의 보호효과

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# Kynurenic acid (KYNA) attenuates 1-metyl-4-phenylpyridinium ion ($MPP^+$)- induced dopaminergic neuronal cell death

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## Objectives

Kynureic acid (KYNA), a metabolite of tryptophan in kynurenine pathway, 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 KYNA exerts protective effect againdt 1-methyl-4-phenylpyridium ($MPP^+$), a causative agent of Parkinson's disease (PD), using SH-SY5Y dopaminergic neuronal cells.

## Materials and Methods

***Cell culture*** SK-SY5Y 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 $MPP^+$ ***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 [(test sample count)-(blank count)/(untreated control count)-(blank count)]100 (Shimoke and Chiba, 2001).

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***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 * 106 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 ul 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 ($\Delta\psi_m$)*** The changes in mitochondrial membrane potential ($\Delta\psi_m$) 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 $\Delta\psi_m$, 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 $\Delta\psi_m$ 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 SH-SY5Y cells with $MPP^+$ induced neuronal cell death and interestingly, pre-treatment of KYNA increased cell viabilities against MPP+. Furthermore, $MPP^+$-induced cell death was proceeded by increased of BAX expression and mitochondrial dysfunction such as collapse of mitochondrial membrane potential ($\Delta\psi m$), release of cytochrome C from mitochondria into cytoplasm and increase of caspase -9/-3 activities. Also, we identified that KYNA reduced significantly the Bax expression levels, mitochondrial dysfunction and caspase activities, eventually inhibited the cell death. Our results implicated KYNA plays a protective role by down- regulating Bax expression level and persisting mitochondrial function in MPP+ - induced neuronal cell death.