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아밀로이드베타로 유도된 신경세포 사멸에 대한 열충격단백질90의 보호 효과

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HSP90 Attenuates β-amyloid-induced Neuronal Cell Death.

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Objectives

In the present study, we determined the protective mechanism of HSP90 against neuronal cell death induced by Aβ.

For the evaluation of protective role of HSP90, we used human neuroblastoma SK-N-SH cell lines, examined AlamarBlue assay, Western blot analysis and immunofluorescence assay.

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.

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.

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

The results may be summarized as follows:

1. In this study, we determined the role of HSP 90 in Ab induced neuronal cell death.
2. HSP 90 attenuated significantly neuronal cell death induced by Ab, followed by protecting mitochondrial damage.
3. Our results showed translocation of HSP 90 into mitochondria in mild stress. Moreover, HSP 90 in mitochondria inhibited decrease of bcl-2 expression in neuronal cell death.
4. HSP90 as chaperone protein significantly attenuates neuronal damage and protects neuroanl cells from neurotoxin such as Aβ.