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Phosphatidylinositol-3-kinase activation blocks amyloid beta-induced neurotoxicity

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Abstract

The phosphatidylinositol-3-kinase (PI3-K) pathway has been suggested to play a pivotal role in neuronal survival. Although PI3-K has been recently identified as a neuroprotectant, there are no reports regarding the effect of a direct PI3-K activator on Aβ-induced neurotoxicity. We investigated whether direct PI3-K activation prevents Aβ-induced neurotoxicity. To evaluate the effect of Aβ on neuronal cells, we treated primary cultured cortical neurons with several doses of Aβ for 72 h. To investigate the protective effect that PI3-K activation has on Aβ-induced neurotoxicity, cells were simultaneously treated with several doses of a PI3-K activator for 72 h. An MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, trypan blue staining, and DAPI staining showed that Aβ decreased neuronal cell viability in a concentration-dependent manner and also that PI3-K activation effectively prevented Aβ-induced neuronal cell death. Aβ significantly decreased survival signals, including phosphorylated Akt, glycogen synthase kinase-3β, and heat shock transcription factor-1. Aβ also increased death signals, such as phosphorylated tau (pThr231) and activated caspase-3. Treatment with a PI3-K activator restored the survival signals and inhibited the death signals. These results suggest that the neurotoxic effect of Aβ can be partially prevented by PI3-K activation.

Keywords: Amyloid beta; Phosphatidylinositol-3-kinase; Neurotoxicity; Alzheimer's dementia

1. Introduction

Alzheimer's disease (AD) is the most common dementia disorder and is characterized by widespread neurodegeneration, deposition of extracellular amyloid beta peptide (Aβ), and formation of intraneuronal neurofibrillary tangles (NFTs) in the brain (Jellinger and Bancher, 1998; Jellinger, 2002; Trojanowski and Lee, 2000). Aβ has been emphasized as a key molecule in AD pathogenesis (Verdile et al., 2004). The amyloid cascade hypothesis suggests that increased Aβ42 results in aggregation, causing inflammatory response, oxidative stress, altered central nervous system homeostasis, neuronal cell death, and dementia (Robinson and Bishop, 2002; Verdile et al., 2004).

Recent studies have proposed that neuroprotectants can prevent Aβ-induced neuronal death by altering several intracellular signaling pathways, including the phosphatidylinositol 3-kinase (PI3-K) pathway (Wei et al., 2002). For example, trophic factors, such as nerve growth factor, vascular endothelial growth factor, and insulin-like growth factor-1 (IGF-1), can activate PI3-K

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(Crowder and Freeman, 1998; Li et al., 2003; Philpott et al., 1997). Activated PI3-K then phosphorylates the serine/threonine kinase Akt (protein kinase B), the main PI3-K downstream effector (Klippel et al., 1998; Koh et al., 2003; Stokoe et al., 1997). The PI3-K signaling pathway plays a central role in neuronal survival (Crowder and Freeman, 1998; Koh et al., 2004; Philpott et al., 1997). These findings indicate that the PI3-K signaling pathway plays a pivotal role in preventing Aβ-induced neuronal cell death, and therefore we hypothesize that direct PI3-K activation could be a potential neuroprotective strategy in AD. Furthermore, there have been no reports on the effect of direct PI3-K activation on Aβ neurotoxicity.

In the present study, we investigated the effect of PI3-K activation on primary cultured cortical neuron death induced by Aβ42.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM, high glucose) was purchased from GIBCO. Drugs and sources are as follows: amyloid beta-protein (1–42) (Aβ42), trypan blue solution, insulin, and DNase I were purchased from Sigma. The PI3-K activator was purchased from Santa Cruz Biotech, Delaware, CA, USA. The PI3-K activator is a peptide with the sequence KKHTDDGYMPMSPGVA, and a molecular weight of 1732.8 Da. The tyrosine phosphorylated version of this peptide binds to the PI3-Kinase SH2 domain and activates the enzyme (Garcia et al., 1993; Rordorf-Nikolic et al., 1995; Shoelson et al., 1992). Before use, these drugs were dissolved in distilled water and further diluted with culture medium to yield the desired final concentrations. To investigate whether the effects seen were due to direct PI3-K activation, cells were treated with 100 μM LY294002 (PI3-K inhibitor; Sigma, Saint Louis, MO, USA) along with the activator; cell viability was then evaluated.

2.2. Cell culture (primary cultured cortical neuron) and treatment

All animal procedures were performed in accordance with the Hanyang University guidelines for the care and use of laboratory animals, and all procedures were approved by the Institutional Review Board of Hanyang University.

Primary cultures were obtained from the cerebral cortex of fetal Sprague–Dawley rats (16 days of gestation) (Meloni et al., 2001). Briefly, rat embryos were decapitated, and the brains were rapidly removed and placed in a Petri dish with ice-cold Hank's balanced salt solution (HBSS; 137 mM NaCl, 5.4 mM KCl, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 5.6 mM glucose, and 2.5 mM Hepes) (Gibco BRL, NY, USA). Single cells dissociated from the whole cerebral cortex were plated on poly-L-lysine (Sigma, Saint Louis, MO, USA) pre-coated 100 mm dishes (Corning) (5×10^6 cells/cm^2) or glass cover slips in 6- or 24-well plates (Nunc) (5×10^5, 2.5×10^6 cells/cm^2). Cultures were incubated in Dulbecco's modified Eagle's medium (high glucose) supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin streptomycin, 3.7 g/l $NaHCO_3$, 5 μg/ml insulin, and *p*-aminobenzoic acid for 41 h. Cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere. Two days after plating on the 4th day in vitro (DIV), cultures were fed with neurobasal medium (2% B27, 1.6% fetal bovine serum, 0.4% horse serum) containing cytosine ararbinofuranoside (1 lM; Sigma) to inhibit non-neuronal proliferation. Only mature cultures (7 days in vitro) were used for experiments.

Different Aβ42 concentrations were tested to assess the effect of Aβ42 on neuronal cell viability. Primary cultured cortical neurons (1×10^5 cells/well) were incubated with Aβ42 [0 (control), 5, 10, 20, and 50 μM] (Sigma) for 72 h (Morishima et al., 2001). Each plate was washed, and cell viability was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and trypan blue stain.

To evaluate the effect of PI3-K activation on Aβ42-induced neurotoxicity, cortical cells (1×10^5 cells/well) were incubated in the presence of 20 μM Aβ42 with several PI3-K activator concentrations (0, 10^1, 10^2, 10^3, 10^4 and 10^5 ng/ml) for 72 h.

Cells in several conditions were harvested 72 h after treatment and immediately used for Akt, phosphorylated Akt, GSK-3β, phosphorylated GSK-3β (Ser9), heat shock transcription factor-1 (HSTF-1), phosphorylated tau (pThr231), and activated caspase-3 immunoblotting to estimate changes in intracellular signaling (Morishima et al., 2001).

2.3. MTT assay and trypan blue staining measure cell viability

MTT is absorbed into cells and transformed into formazan by mitochondrial succinate dehydrogenase. Formazan accumulation directly reflects mitochondrial activity, which is an indirect measure of cell viability. Cells were plated at a density of 1×10^4 cells/well in a 96-well plate, cultured, and differentiated prior to adding 50 μl of 2 mg/ml MTT (Sigma) to 200 μl of medium in each well. For each well, 220 μl of resting solution was removed and 150 μl of dimethyl sulfoxide was added. The precipitate in each well was resuspended on a microplate mixer for 10 min, and an optical density (OD) reading at 540 nm was measured using an ELISA plate reader. All results were normalized to OD values for an identical condition with no cells (Koh et al., 2005). For trypan blue staining, 10 μl of trypan blue solution was incubated for 2 min with 10 μl of cells from each sample. Unstained live cells were counted on a hemacytometer (Spencer, Buffalo, NY, USA) (Koh et al., 2005).

2.4. DAPI staining to assess apoptosis

DAPI staining (Jiang et al., 2000) was performed as follows to evaluate apoptosis. Cortical cells were incubated for 72 h with one of three treatments: (1) neither Aβ42 nor PI3-