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Research report

Zinc-induced anti-apoptotic effects in SH-SY5Y neuroblastoma cells via the extracellular signal-regulated kinase 1/2

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Abstract

Zinc levels are increased in brain areas severely affected by Alzheimer's disease (AD) pathologies. Zinc has both protective and neurotoxic properties and can stimulate both phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. Several kinases related to these pathways including protein kinase B (PKB), p70 S6 kinase (p70S6K), and extracellular signal-regulated kinase 1/2 (ERK1/2) are known cell survival factors and are overactivated in neurons bearing neurofibrillary tangles (NFTs) in AD. The present study aimed to determine whether anti-apoptotic effects of zinc are mediated via these signaling pathways. Zinc was used to treat SH-SY5Y neuroblastoma cells and effects investigated in relation to PKB, p70S6K, and ERK1/2 in the absence and presence of the pro-apoptotic agent staurosporine (STS). Cell damage was evaluated by measuring levels of DNA fragmentation as well as the WST-1 assay for cell viability. Results indicated that: (1) treatment with high doses of zinc (≥400 μM) for short time periods (≤2 h) gave rise to increased levels of DNA fragments, increased cell membrane permeability, and reduced mitochondria membrane potential; (2) treatment with 100 μM zinc for >2 h reversed an increased DNA fragmentation due to U0126 inhibition of ERK1/2; (3) increased DNA fragmentation due to STS could be protected against by 100 μM zinc; (4) the protective effects of 100 μM zinc on STS-induced DNA fragmentation could be partially reversed by U0126. These results indicate that a zinc-induced anti-apoptotic response in SH-SY5Y cells likely occurs through ERK1/2.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Alzheimer's—miscellaneous

Keywords: Zinc; DNA fragmentation; Cell viability; ERK1/2; Alzheimer's disease

1. Introduction

Zinc has been studied for decades since it is fundamental to the structure and function of many proteins [8]. Maintenance of discrete subcellular pools of zinc contributes to a number of important biological processes including gene expression, DNA synthesis, enzymatic catalysis, hormonal storage and release, neurotransmission, and memory. We are interested in zinc because levels of this ion are increased in brain areas severely affected by Alzheimer's disease (AD) pathologies [7].

Zinc has been found to activate both phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways [19,30]. Kinases such as protein kinase B (PKB) and extracellular signal-regulated kinase 1/2 (ERK1/2) in these two pathways, and p70 S6 kinase (p70S6K), are known to modulate cell survival [6,14,25]. In addition, both PKB and ERK1/2 have been shown to phosphorylate tau at some sites to a similar extent seen in AD brain [16,21,35]. Activated forms of PKB, p70S6K, and

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ERK1/2 have been found to be accumulated in neurons bearing neurofibrillary tangles (NFTs), in particular those neurons prior to developing tangles [1,32,33]. Zinc could enhance Aβ aggregation that in turn induces tau hyperphosphorylation [4,12]. Thus, zinc may influence tau phosphorylation through Aβ pathways or by directly activating tau candidate kinases.

There is a big controversy regarding the role of zinc in cell death. Zinc was reported to induce both necrosis and apoptosis [13]. Zinc protects against apoptosis induced by diverse physical, chemical, or immunologic stimuli in cultured cells of lymphoid, hepatic, or neoplastic origin by preventing endonucleosomal fragmentation and subsequent cytolysis [43]. Pre-administration of zinc by subcutaneous injection significantly reduced the nuclear damage and subsequent neuronal death in hippocampus induced by transient global ischemia in Mongolian gerbils [26]. Thus, zinc involvement in AD neuronal loss needs to be further investigated. One hypothesis is that zinc promotes neuronal survival and leads neurons into neurofibrillary degeneration instead of apoptosis [27,31].

Staurosporine (STS) is a general protein kinase inhibitor that can induce apoptosis in a wide variety of cell types, such as embryonic stem cells and SH-SY5Y cells [15,34]. STS treatment could impair mammalian target of rapamycin (mTOR) signaling and lead to p70S6K dephosphorylation and inactivation in Swiss 3T3 cells, which occur before apoptosis execution [44]. Thus, the possible role of zinc and its mechanism in cell death was investigated in SH-SY5Y cells, in which cell damage was evaluated by measuring levels of cytosolic DNA fragments as an index of apoptosis. In addition, the WST-1 assay was used as a measure of general cell viability. In initial experiments, zinc toxicity was also studied using propidium iodide and rhodamine 123 staining. The aim was to determine whether anti-apoptotic effects of zinc are mediated via PI3K and MAPK signaling pathways.

2. Materials and methods

2.1. Materials

Propidium iodide (PI), rhodamine 123 (Rh123), protease inhibitor cocktail, rapamycin, staurosporine (STS), and zinc sulphate were purchased from Sigma-Aldrich (St. Louis, MO). The cell proliferation reagent WST-1 and the cell death ELISA ^{plus} kit for fragmented DNA were from Roche Molecular Biochemicals (Mannheim, Germany). Fetal bovine serum (FBS), F12/DMEM culture media were from Invitrogen AB (Täby, Sweden). Wortmannin, U0126, affinity purified polyclonal rabbit antibodies against p70S6K (T389 or T421/S424), ERK1/2 (T202/Y204), and PKB (T308) were purchased from Cell Signaling Technology (Beverly, MA). Antibody to PKB (S473) was bought from Biosource International (Camarillo, CA).

2.2. Cell culture, treatment, and preparation of cell extracts for Western blotting

SH-SY5Y human neuroblastoma cells were cultured in F12/DMEM (1:1) media supplemented with 5% (v/v) FBS, 100 units/ml penicillin, and 0.1 mg/ml streptomycin in a humidified atmosphere of 5% CO_2/95% air at 37 °C. The cells were then cultured in 0.5% FBS media for 2 days, and prior to treating the cells with zinc, the cultures were kept in fresh serum-free medium for 2 h (serum withdrawal condition). Control experiments were also performed using cells that were kept cultured in 5% FBS (non-stressed, serum control condition). The cells were harvested and lysed in buffer containing 2 mM EGTA, 25 mM NaF, 200 μM Na_3VO_4, 0.5 mM PMSF, 5 mM EDTA, 150 mM NaCl, 50 mM Tris–HCl (pH 7.4), 1% Triton X-100, and protease inhibitor cocktail (1:200). The cell lysates were then kept at −80 °C. Protein concentration was determined with the BCA kit.

2.3. Double-fluorescent confocal microscopy

SH-SY5Y cells were seeded on FALCON® 8-well culture slides. At the indicated time points of zinc treatment, cells were stained with the fluorescent dye rhodamine 123 (Rh123) (10 μM) for 30 min or propidium iodide (PI) (5 μg/ml) for 15 min. A Laser Scanning Confocal Imaging System (Radiance Plus, Bio-Rad, Hercules, CA) was employed to acquire the images. The system is equipped with a Nikon Eclipse inverted microscope (TE300). An argon ion laser that excites at 488 nm with a dichroic beamsplitter 560DCLP and a band-pass filter HQ515/30 was used to detect the Rh123 signal. A Diode laser that excites at 547 nm with an HQ590/70 emission filter was used to detect the PI signal.

2.4. Western blotting

Following the method described previously [1], the samples were subjected to 12% SDS-polyacrylamide gel electrophoresis and separated proteins transferred to nitrocellulose membranes. Membranes were blocked with 5% fat-free milk for 30 min, incubated with primary antibodies overnight at 4 °C, and anti-rabbit secondary antibody conjugated to horseradish peroxidase for 1 h. Bound antibodies were detected with ECL™ Western blotting detection reagents (Amersham Pharmacia Biotech, England).

2.5. DNA fragmentation assay

Using mouse monoclonal antibodies against DNA and histone, the cell death detection ELISA ^{plus} kit was used to measure the cytosolic fraction of mono- and oligonucleosomes (DNA fragments). The SH-SY5Y cells were grown in 96-well plates. After treatment, the plates were centrifuged