Original Article

Cadmium Activates Reactive Oxygen Species-dependent AKT/mTOR and Mitochondrial Apoptotic Pathways in Neuronal Cells*

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Abstract

Objective To examine the role of Cd-induced reactive oxygen species (ROS) generation in the apoptosis of neuronal cells.

Methods Neuronal cells (primary rat cerebral cortical neurons and PC12 cells) were incubated with or without Cd post-pretreatment with rapamycin (Rap) or N-acetyl-L-cysteine (NAC). Cell viability was determined by MTT assay, apoptosis was examined using flow cytometry and fluorescence microscopy, and the activation of phosphoinositide 3'-kinase/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) and mitochondrial apoptotic pathways were measured by western blotting or immunofluorescence assays.

Results Cd-induced activation of Akt/mTOR signaling, including Akt, mTOR, p70 S6 kinase (p70 S6K), and eukaryotic initiation factor 4E binding protein 1 (4E-BP1). Rap, an mTOR inhibitor and NAC, a ROS scavenger, blocked Cd-induced activation of Akt/mTOR signaling and apoptosis of neuronal cells. Furthermore, NAC blocked the decrease of B-cell lymphoma 2/Bcl-2 associated X protein (Bcl-2/Bax) ratio, release of cytochrome c, cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP), and nuclear translocation of apoptosis-inducing factor (AIF) and endonuclease G (Endo G).

Conclusion Cd-induced ROS generation activates Akt/mTOR and mitochondrial pathways, leading to apoptosis of neuronal cells. Our findings suggest that mTOR inhibitors or antioxidants have potential for preventing Cd-induced neurodegenerative diseases.

Key words: Cadmium; Apoptosis; AKT/mTOR pathway; Mitochondrial apoptotic pathway; Primary rat cerebral cortical neurons; PC12 cells

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INTRODUCTION

Cd is a metal frequently used in various industrial activities such as battery and alloy manufacturing, and it is a ubiquitous environmental contaminant present in tobacco smoke and food[1-2]. It is a toxic metal capable of damaging several organs, including the brain[3-4]. Previous studies have shown that Cd-induced neurotoxicity is a result of reactive oxygen species (ROS) generation, which leads to oxidative stress[5-8]. Under pathological conditions, excessive amounts of Cd-induced ROS can activate related signaling pathways, resulting in apoptosis of neuronal cells[6,9].

The mammalian target of rapamycin (mTOR), a 289-kD Ser/Thr kinase, lies downstream of protein kinase B (Akt/PKB) and regulates cell proliferation, growth, and survival[10]. Activation of Akt may positively regulate mTOR, leading to increased phosphorylation of p70 S6 kinase (p70 S6K) and eukaryotic initiation factor 4E binding protein 1(4E-BP1), the two best-characterized downstream effector molecules of mTOR[10-11]. Cd-induced ROS generation has been reported to be related to activation of the mTOR pathway in neuronal model cells such as rat pheochromocytoma (PC12) and human neuroblastoma (SH-SY5Y) cell lines[7]. This prompted us to examine whether Cd activates mTOR by inducing ROS in primary neurons.

Mitochondria have been shown to play a central role in apoptosis[12]. Mitochondrial damage results in the release of pro-apoptotic proteins such as cytochrome c (Cyt C) and apoptosis-inducing factor (AIF), which trigger caspase-dependent or caspase-independent cell death[13]. Recently, we demonstrated that Cd-induced apoptosis is partially associated with the activation of caspase-dependent and caspase-independent mitochondrial signaling pathways in primary neurons and PC12 cells[14-15]. Excess generation of ROS might contribute to mitochondrial damage and cause cell death by triggering endogenous apoptotic cascade reactions[16]. However, little is known about the role of ROS in Cd-mediated activation of mitochondrial signaling pathways in neuronal cells.

Here, we show that Cd induces ROS generation, which is correlated with activation of the Akt/mTOR pathway, that in turns leads to neuronal apoptosis. We also show that Cd-induced generation of ROS activates caspase-dependent and caspase-independent mitochondrial signaling pathways, which leads to neuronal apoptosis.

MATERIALS AND METHODS

Reagents

NEUROBASAL™ Medium and B27 Supplement were purchased from Invitrogen (Grand Island, NY, USA). Fetal calf serum (FCS) was obtained from Hyclone Laboratories (Logan, UT, USA). Dulbecco's modified Eagle's medium (DMEM)-F12 (1:1), cadmium acetate ($CdAc_2$), 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), Poly-D-lysine (PDL), Hoechst 33258, rapamycin (Rap), and penicillin/streptomycin were purchased from Sigma-Aldrich (Saint Louis, MO, USA). RPMI 1640 medium and horse serum were supplied by Life Technologies (Grand Island, NY, USA). The Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit was obtained from BD Biosciences (San Diego, CA, USA). Antibodies against Akt, phospho-Akt (Thr308), mTOR, phospho-mTOR (Ser2448), p70 S6K, phospho-p70 S6K (Thr389), phospho-4E-BP1 (Thr37/46), cleaved caspase-3, cleaved poly (ADP-ribose) polymerase (PARP), cytochrome c (Cyt C), B-cell lymphoma 2 (Bcl-2), Bcl-2 associated X protein (Bax), β-actin, and cyclooxygenase (COX)-IV were obtained from Cell Signaling Technology (Boston, MA, USA). Antibodies against apoptosis-inducing factor (AIF) and endonuclease G (Endo G) were obtained from Abcam (Cambridge, MA, USA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Tetramethylrhodamine (TRITC)-conjugated AffiniPure goat anti-rabbit IgG and FITC-conjugated AffiniPure goat anti-rabbit IgG were obtained from Bioworld Technology (Minneapolis, MN, USA). Enhanced chemiluminescence solution was obtained from Thermo Fisher Scientific (Waltham, MA, USA). All other reagents were of analytical grade.

Ethics Statement

Fetal Sprague-Dawley rats at 18-19 d of gestational age were obtained from the Laboratory Animal Center of Yangzhou University (Yangzhou, China). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Research Council. The Animal Care and Use Committee of Yangzhou University approved all experiments and procedures conducted in the animals [approval ID: SYXK (Su) 2007-0005].