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Cytoprotective effects of selenium on cadmium-induced LLC-PK_1 cells apoptosis by activating JNK pathway

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Abstract

Extensive studies have indicated that the apoptosis pathway appears to be associated with intracellular reactive oxygen species (ROS) production in cadmium-induced nephrotoxicity, however, the precise cellular mechanism remains unclear. The purpose of this study was to determine the relationships between the activation of phosphorylated c-jun *N*-terminal kinase (JNK) and cadmium-induced apoptosis, and assess the possible cytoprotective mechanism of selenium. Our study clearly revealed cadmium treatment caused apoptosis in LLC-PK_1 cells, which was partially suppressed by pretreatment with selenium, an antioxidant nutrient. Further studies found the phosphorylation of JNK kinase increased with exposure to cadmium for 3 h, even remained elevated at 9 h in the time course study, and the activation of phosphorylated JNK was detected in a dose-dependent manner. In addition, a concomitant time-dependent increase in caspase-3 activities was observed by cadmium treatment. During the process, selenium played the same role as *N*-acetyl-L-cysteine (NAC), a free radical scavenger. Pretreatment of cells with selenium partially suppressed of the phosphorylation of JNK, coupled with caspase-3 activation involved in cadmium-induced apoptosis. In conclusion, our studies provided a molecular linkage between the phosphorylation of JNK and cadmium-induced LLC-PK_1 cells apoptosis, and demonstrated selenium also contributed a potentially protection to prevent cadmium-cytotoxicity.

Keywords: Cadmium; Apoptosis; Selenium; JNK; Caspase-3

1. Introduction

Cadmium is a widespread environmental and industrial pollutant, which is classified by International Agency for Research on Cancer (IARC) as Group I carcinogen to humans (IARC, 1993). It has been well established that chronic exposure to cadmium causes irreversible kidney damage and renal tubular dysfunction (Nishijo et al., 2006; Horiguchi et al., 2006). Despite being one of the major routes for cadmium absorption, the toxic mechanism of cadmium on renal tissue is still poorly understood.

During the last decade, a number of studies have shown that cadmium induces apoptosis of the proximal tubular cells (Lee et al., 2005; Ishido et al., 1998). Although extensive research has been undertaken to elucidate signal pathways in apoptosis, at present, oxidative stress has been considered an important possible mechanism of cadmium toxicity (Kim and Sharma, 2006; Filipic et al., 2006). Accumulated evidence has also shown that cadmium increased cellular reactive oxygen species (ROS) levels (Valko et al., 2006; Pathak and Khandelwal, 2006a, b; Oh and Lim, 2006), lipid peroxidation and alteration in glutathione (GSH) levels in many cell types (Pathak and Khandelwal, 2006a,b; Shaikh et al., 1999), suggesting that cadmium-induced apoptosis may be connected with oxidative stress. Our previous studies showed that cadmium

Abbreviations: $CdCl_2$, cadmium chloride; Na_2SeO_3, sodium selenite; JNK, c-Jun N-terminal kinase; SAPK, stress-activated protein kinase; MAPK, mitogen-activated protein kinases; ROS, reactive oxygen species; GSH, glutathione; NAC, *N*-acetyl-L-cysteine; PBS, phosphate buffered saline; FITC, Annexin V-fluorescein isothiocyanate; PI, propidium iodide.

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can induce apoptosis in LLC-PK$_1$ cells, increased cellular ROS production (data not shown), and that selenium has a protective effect against cadmium cytotoxicity (Ren et al., 2004). Nevertheless, the exact mechanism in oxidative stress of selenium protective effect against cadmium-induced apoptosis has not been clarified.

The c-jun N-terminal kinase (JNK), also known as stress-activated protein kinase (SAPK), belongs to the mitogen-activated protein kinases (MAPK) superfamily. Based on substantial evidence, JNK signaling is also sensitive to a decreased or increased oxidative environment (Gomez et al., 1996; Wang et al., 1998), which is frequently involved in mediating stress responses induced from a diverse array of factors (Barr and Bogoyevitch, 2001; Pearson et al., 2001). In response to various environmental stresses including heavy metals, the c-jun N-terminal kinase (JNK) is activated by dual phosphorylation on Thr183 and Thr185 (Derijard et al., 1994; Kyriakis and Avruch, 1996). Resent evidence suggests that JNK activity may play an important role in triggering apoptotic signaling (Chen et al., 1996; Eilers et al., 1998). For example, studies indicated that the JNK/c-jun signaling cascade plays a crucial role in cadmium-induced neuronal cell apoptosis and apoptosis in CL3 human lung adenocarcinoma cells (Chuang et al., 2000; Kim and Sharma, 2004). In contrast to the above reports, Lag et al., (2005) demonstrated the MAPK p38, more than JNK pathway seemed to be involved in the cadmium-induced apoptosis in Clara cells and type 2 cells. Considering the above results, JNK signaling pathway may be responsible for cadmium-induced apoptosis in LLC-PK$_1$ cells and the relationships between protective mechanism of selenium against cadmium-induced apoptosis and JNK pathway activation need further characterization.

In order to gain further insights into the toxic mechanism response to cadmium, the objectives of this study were to examine the relationships between the phosphorylation of JNK and cadmium-induced apoptosis in LLC-PK$_1$ cell lines and to explore the protective mechanism of selenium.

2. Materials and methods

2.1. Materials

Cadmium chloride (Wako PureChemical Ind., Osaka, Japan) and Sodium selenite (Shanghai Zhenxin Chemical CO., China) were dissolved in water, sterilized with 0.22 μm filters, and added to cultures at the indicated time and concentrations. Cell culture reagents were obtained from GIBCO Life Technology (Grand Island, NY, USA). Antibodies specific for the total and phospho-SAPK/JNK (Thr183/Tyr185), procaspase-3 and anti-rabbit IgG, HRP-linked antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). β-Actin antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Hoechst 33258 Staining and caspase-3 activity kit were obtained from Beyotime Institute of Biotechnology (Haimen, China), and Annexin V-FITC Apoptosis Detection kit was purchased from Pharmingen (Becton Dickinson Company, San Jose, CA, USA). *N*-Acetyl-L-cysteine (NAC) and other reagents in the molecular studies were supplied from Sigma (St. Louis, MO, USA).

2.2. Cell culture

LLC-PK$_1$ cells, a porcine renal epithelial cell line, were generously provided by Dr. Xiao-Ming Zhou (Uniformed Services University, USA). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin G, 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. For each experiment, exponentially growing LLC-PK$_1$ cells were plated at 5×10^4 cells/well in 6-well culture plates, cultured for 1 day, and maintained in serum-free medium for 12 h before being used for subsequent experiments.

2.3. Hoechst 33258 staining

Apoptotic morphological changes in the nuclear chromatin of cells were detected by Hoechst 33258 staining. LLC-PK$_1$ cells were seeded on sterile cover glasses placed in the 6-well plates. After overnight growth, cells were pre-treated with NAC (500 μM) or selenium (20 μM) for 30 min, then treated with 40 μM cadmium for 12 h, cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 10 min, and then incubated with 50 μM Hoechst 33258 staining solution for 10 min. After three washes with PBS, the cells were viewed under a fluorescence microscope (Olympus, IX-70, Japan).

2.4. Flow cytometric assessment of apoptosis

The measurement of phosphatidylserine redistribution in a plasma membrane was conducted according to the protocol outlined by the manufacturer of the Annexin V-FITC Apoptosis Detection kit (Becton Dickinson Company, San Jose, CA, USA). Briefly, After pre-treatment with NAC (500 μM) or selenium (20 μM) for 30 min, then with 40 μM cadmium for 12 h, harvested cells were suspended in a binding buffer (1×). An aliquot of 100 μl was incubated with 5 μl of Annexin V-FITC and 5 μl of PI for 15 min in dark, and 400 μl binding buffer (1×) was added to each sample. The stained cells were analyzed directly by flow cytometry using the Cell Quest program (Becton Dickinson, Franklin, NJ).

2.5. Western blotting

Cells were grown at 5×10^4 cells/well in 6-well microplates and incubated with NAC, selenium and cadmium for indicated time. Following treatment, cells were washed with PBS, and total cells were prepared by scraping in 200 μl of lysis buffer [20 mM Tris–HCl (pH 8.0), 1 mM sodium ortho-