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The role of Nrf2 in protection against Pb-induced oxidative stress and apoptosis in SH-SY5Y cells

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ABSTRACT

Lead exerts severe adverse effects on the nervous system in which oxidative stress might mediate impairments. In this study, we focused on Nrf2, which has been identified to significantly influence the protection of a cellular system against many xenobiotic compounds. We found that PbAc exhibited neurotoxicity mainly through oxidant-based processes and could be inhibited by NAC and DPI in SH-SY5Y cells. As a defense response, Nrf2 was activated when exposed to PbAc, thereby inducing a rapid increase in Nrf2 nuclear accumulation, as well as Nrf2-ARE binding activities in a ROS-dependent manner. Analysis of Nrf2-regulated gene expression and protein showed that PbAc could induce the mRNA transcription of HO-1, GSTα1, GCLM, GCLC, and NQO1, as well as the protein expression of HO-1 and γ-GCS. The responses of these genes to PbAc were regulated by Nrf2. Silencing Nrf2 expression in SH-SY5Y cells inhibited PbAc-induced gene transcription and protein expression. Overexpression of Nrf2 led to decreased ROS production and cell apoptosis, as well as increased cell viability under PbAc exposure. These results indicated that the Nrf2-ARE system exhibited a protective role in Pb-induced neurotoxicity, providing potential therapeutic strategies for the prevention and treatment of Pb-related diseases.

1. Introduction

Lead (Pb), a ubiquitous environmental and industrial pollutant, can be detected in almost all phases of environmental and biological systems. The wide use of lead has caused air, water, and soil contamination worldwide (Needleman, 2004). Lead also induces various physiological, biochemical, and behavioral dysfunctions in animals and human beings, primarily targeting the central nervous system (Basha et al., 2003; Chetty et al., 2001; Hu et al., 2014; Wilson et al., 2000). Exposure to a low dose of PbAc leads to subtle nonspecific disorders in brain functions, such as impaired cognition, impaired hearing and sight, reduced perception, and disorders in neurobehavioral functioning, including aggression (Baranowska-Bosiacka et al., 2013; Bleecker et al., 2005).

Numerous studies on lead-induced neurologic impairment have been conducted; however, the precise mechanisms by which lead exerts neurotoxic effects remain poorly understood. Several studies have identified oxidative stress as the primary contributory agent in the pathogenesis of lead exposure. Oxidative stress has been implicated to affect specific organs, including brain, liver, kidney

Abbreviations: ARE, antioxidant response element; BSO, buthionine sulfoximine; DCFH-DA, 2′7′-dichlorodihydrofluorescein diacetate; DMSA, dimercaptosuccinic acid; DPI, diphenyliodonium chloride; EMSA, Electrophoretic mobility shift assay; FBS, Fetal Bovine Serum; GCLC, glutamyl cysteine ligase catalytic units; GCLM, glutamyl cysteine ligase modulatory; GSTα1, Glutathione S Transferase Alpha 1; GST Ya, Glutathione S transferase Ya; HBSS, Hank's Balanced Salt Solution; HO-1, Heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; LPS, lipopolysaccharide; MFI, mean fluorescence intensity; MRP1, multidrug-resistance protein; NAC, *N*-acetyl-L-cysteine; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, NF-E2-related factor 2; PbAc, lead acetate; PBS, phosphate-buffered saline; ROS, reactive oxygen species; t-BHQ, tert-butylhydroquinone; TNF, tumor necrosis factor; γ-GCS, γ-glutamylcysteine synthetase; 3MP-ITC, 3-Morpholinopropyl isothiocyanate.

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tissue, lung, endothelial tissue, testes, and sperm associated with lead (Hsu and Guo, 2002). The mechanisms of lead toxicity leading to free radical damage involve two separate but related pathways: the generation of ROS and direct depletion of antioxidant reserves (Devi et al., 2007). The first pathway includes hydroperoxides, singlet oxygen, and hydrogen peroxide. Lead-induced oxidative stress can contribute to the pathogenesis of lead poisoning by disrupting the pro-/antioxidant balance in cells (Bokara et al., 2008). This event prompts cell apoptosis through mitochondrial apoptotic pathways by decreasing the inner mitochondrial membrane potential (Marchlewicz et al., 2009). This reduction leads to the imbalance of Bax/Bcl-2, thereby releasing cytochrome c into the cytoplasm and activating caspase-3 enzymes (Chetty et al., 2005; He et al., 2000). Oxidative stress also causes DNA alterations, including fragmentation, rearrangements, deletions, and point mutation (Ercal et al., 2001). Thus, lead-induced production of ROS in cells can damage cells.

Self-defense mechanisms against external stimulation, including lead exposure, exist in cells. Among these mechanisms is NF-E2-related factor 2 (Nrf2), a redox-sensitive transcription factor belonging to the Cap 'N' collar family. Nrf2 participates in the regulation of antioxidant response element (ARE)-mediated gene expression and has been identified as a key regulator of inflammation (Ma, 2013). Under normal conditions, Nrf2 is present in the cytoplasm in association with Kelch-like ECH-associated protein 1 (Keap1), which negatively regulates Nrf2 by retaining it in the cytosol and enhancing its proteasomal degradation. Upon exposure to electrophiles and ROS, Nrf2 dissociates from Keap1 and translocates into the nucleus, where it dimerizes with small Maf binding proteins. Binding of these heterodimers to ARE ultimately activates ARE-dependent gene expression (Kobayashi and Yamamoto, 2005; Li and Kong, 2009; Motohashi and Yamamoto, 2004). These genes include phase II detoxifying antioxidant enzymes, the cytoprotective enzyme heme oxygenase-1 (HO-1), as well as phase II enzymes. Examples of phase II enzymes are NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione S transferase alpha 1 (GSTα1), which detoxify endogenous and exogenous chemicals through reduction and conjugation reactions. Glutamyl cysteine ligase modulatory (GCLM) unit and glutamyl cysteine ligase catalytic (GCLC) unit, the two subunits of the rate-limiting enzyme in glutathione biosynthesis, are regulated by Nrf2 (Lee et al., 2005; Maher et al., 2007; Rushworth et al., 2008, 2011). Nrf2 knockout in mice substantially increases the susceptibility of mice to a broad range of chemical toxicity and disease conditions associated with oxidative pathology (Kensler et al., 2007; Rangasamy et al., 2005). Meanwhile, pharmacological boosting of Nrf2 activity with chemoprotective agents protects animals from oxidative damage. Induction of ARE-regulated genes is regarded as an important method in studies on chemoprevention. In chemoprevention, phenolic antioxidants, such as butylated hydroxyanisole and tert-butylhydroquinone (t-BHQ), protect animals from chemical carcinogenesis, and protection is correlated with the induction of Nrf2 (Talalay et al., 2003). Thus, the Nrf2 pathway is one of the most important protective mechanisms against oxidative stress in the cell.

Many studies have determined that Nrf2 is activated by environmental or industrial pollutants, such as arsenic and cadmium, and protects against these pollutants (Cordova et al., 2014; Son et al., 2014). However, studies on the relationship between lead exposure and Nrf2 in the neural system are limited. Korashy and El-Kadi (Korashy and El-Kadi, 2006) were the first to identify that Pb^{2+} and Hg^{2+} regulate the expression of the NQO1 and glutathione S transferase Ya (GST Ya) genes through Nrf2/ARE-dependent transcriptional mechanisms. This regulation induces the expression of NQO1 and GST Ya mRNAs in a time-dependent manner in Hepa-1c1c7 cells. Wang et al. (2013) proved the upregulation of Nrf2 and multidrug resistance protein 1 in response to lead-induced oxidative and electrophilic stress in rat testes. However, the function and mechanism of Nrf2 in response to lead remain unknown.

In the present study, we examined the possible protective role of the Nrf2-ARE pathways against oxidative damage induced by PbAc in SH-SY5Y human neuroblastoma cells. SH-SY5Y cells are relevant in vitro model systems for primary neuronal cells because they stop dividing, grow long neurites, and show changes in cellular composition associated with neuronal differentiation in response to treatment with nerve growth factor and retinoic acid (Prins et al., 2010). We explored whether PbAc-induced Nrf2 activation increases Nrf2-ARE-related antioxidant enzyme expression, as well as the mechanism by which PbAc activates Nrf2. The Nrf2 signal transduction pathway was proven to be involved in protecting SH-SY5Y cells under PbAc exposure by overexpression or knockdown of Nrf2.

2. Materials and methods

2.1. Materials

PbAc, NAC, diphenyliodonium chloride (DPI), and buthionine sulfoximine (BSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). DMEM/F12 and Hank's balanced salt solution were purchased from Thermo Scientific. Fetal bovine serum (FBS) was acquired from GIBCO (Invitrogen, Carlsbad, CA, USA). Deionized water was produced using an ultrapure water purification system.

2.2. Cell culture

Human neuroblastoma SH-SY5Y cells were obtained from the China Center for Type Culture Collection (Shanghai, China). SH-SY5Y cells were cultured in DMEM/F12 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS, 1% non-essential amino acids (Hyclone, Thermo Scientific), 100 U/mL penicillin, and 100 μg/mL streptomycin (Hyclone, Thermo Scientific). Cultures were maintained at 37 °C in an atmosphere of 5% carbon dioxide and 95% air in 100% relative humidity.

2.3. Measurement of cell viability

Cell viability was assessed using the Cell Counter Kit-8 (CCK-8) assay (Dojindo Laboratories, Japan) in accordance with the manufacturer's instruction. SH-SY5Y cells were seeded in a 96-well plate at an initial density of 1×10^4 cells/well and then left to attach overnight. After the indicated treatments, 10 μM CCK-8 solution dissolved by a serum-free medium was added to each well of the plate. The cells were incubated for 1 h in the incubator, and the absorbance was quantified on an automated reader (Bio-Tec, CA, USA).

2.4. Annexin V-FITC/PI double staining assay

Lead-induced apoptosis was determined by flow cytometry using the Annexin V-FITC Apoptosis Detection Kit (Biovision, CA, USA) in accordance with the manufacturer's instructions. In brief, SH-SY5Y cells (2.5×10^5 cells/well) were seeded into six-well plates. At the end of the treatment, the cells were collected by centrifugation at 1000 r/min for 5 min and then washed twice with ice-cold PBS. The cells were resuspended in 500 μL of binding buffer and then stained with Annexin V-FITC solution (5 μL) and PI solution (5 μL) for 15 min in the dark at room temperature. The samples were analyzed by flow cytometry (Becton Dickinson, San Jose, CA, USA). A total of 10,000 cells were analyzed for each sample. The