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Arsenic induces reactive oxygen species-caused neuronal cell apoptosis through JNK/ERK-mediated mitochondria-dependent and GRP 78/CHOP-regulated pathways

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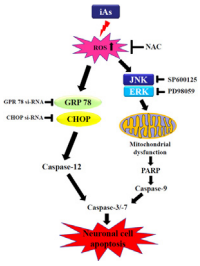
HIGHLIGHTS

- Inorganic arsenic (iAs) induced oxidative stress leading to neuronal cell apoptosis.
- iAs caused JNK/ERK activation-mediated mitochondria-dependent caspase cascades pathway.
- iAs was capable of inducing GRP78 and CHOP activations-regulated apoptotic signals.

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic (As), a well-known high toxic metal, is an important environmental and industrial contaminant, and it induces oxidative stress, which causes many adverse health effects and diseases in humans, particularly in inorganic As (iAs) more harmful than organic As. Recently, epidemiological studies have suggested a possible relationship between iAs exposure and neurodegenerative disease development. However, the toxicological effects and underlying mechanisms of iAs-induced neuronal cell injuries are mostly

Abbreviations: ROS, reactive oxygen species; MAPKs, mitogen-activated protein kinases; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-related kinase; Nrf2, nuclear-factor-E2-related factor 2; PARP, poly (ADP-ribose) polymerase; NAC, *N*-acetylcysteine; ER stress, endoplasmic reticulum stress; GRP, glucose-regulated protein; CHOP, C/EBP homologue protein; XBP-1, X-box binding protein-1; si-RNA, small interference RNA; MMP, mitochondrial membrane potential.

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unknown. The present study demonstrated that iAs significantly decreased cell viability and induced apoptosis in Neuro-2a cells. iAs also increased oxidative stress damage (production of malondialdehyde (MDA) and ROS, and reduction of Nrf2 and thioredoxin protein expression) and induced several features of mitochondria-dependent apoptotic signals, including: mitochondrial dysfunction, the activations of PARP and caspase cascades, and the increase in caspase-3 activity. Pretreatment with the antioxidant N-acetylcysteine (NAC) effectively reversed these iAs-induced responses. iAs also increased the phosphorylation of JNK and ERK1/2, but did not that p38-MAPK, in treated Neuro-2a cells. NAC and the specific JNK inhibitor (SP600125) and ERK1/2 inhibitor (PD98059) abrogated iAs-induced cell cytotoxicity, caspase-3/-7 activity, and JNK and ERK1/2 activation. Additionally, exposure of Neuro-2a cells to iAs triggered endoplasmic reticulum (ER) stress identified through several key molecules (GRP 78, CHOP, XBP-1, and caspase-12), which was prevented by NAC. Transfection with GRP 78- and CHOP-specific si-RNA dramatically suppressed GRP 78 and CHOP expression, respectively, and attenuated the activations of caspase-12, -7, and -3 in iAs-exposed cells. Therefore, these results indicate that iAs induces ROS causing neuronal cell death via both JNK/ERK-mediated mitochondria-dependent and GRP 78/CHOP-triggered apoptosis pathways.

1. Introduction

Arsenic (As), a naturally occurring element in the earth's crust, is a toxic metalloid in the environment and is present in a variety of chemical forms. Inorganic As (iAs) is more toxic than the organic form and the predominant in surface and underground water reservoirs (ATSDR, 2007; Nordstrom, 2002). Arsenic-containing drinking water supplied by natural deposits or from agricultural and industrial practices is the major source of iAs exposure in humans (Chen et al., 2007). Epidemiological studies have demonstrated an association between chronic exposure to arsenic-contaminated water and a wide range of adverse health effects (including various cancers, cardiovascular disease, diabetes mellitus, liver and skin diseases) in Argentina, Bangladesh, Chile, Denmark, India, Mexico, Taiwan, and the United States (Chen and Ahsan, 2004; Das et al., 2012; Ferreccio et al., 2000; Huang et al., 2008; Meliker et al., 2007). Some studies have reported that arsenic causes severe nervous system dysfunction or signs of pathogenesis of neuropathy in humans, such as impairments of learning and deterioration in pattern memory and switching attention (Mathew et al., 2010; Tsai et al., 2003). Early-life exposure to arsenic-contaminated water has been linked to increased mortality and morbidity in infant and cognitive deficits in school-aged children (Hamadani et al., 2011; Rahman et al., 2009; Vahidnia et al., 2007). In experimental animals, arsenic exposure results in brain injuries, which cause behavioral abnormalities, changes in the morphology and structure, and neuronal cell death (Flora et al., 2009). Furthermore, Chattopadhyay et al. (2002) and Dwivedi et al. (2011) reported that arsenic exposure resulted in significant production of reactive oxygen species (ROS), causing oxidative DNA damage, mitochondrial metabolism impairments, and severe pathological changes leading to apoptosis in neuronal cells. Based on these findings, it is raised concern that the brain is an important target of arsenic-induced injuries. However, the cellular and molecular mechanisms underlying arsenic-induced neurotoxicity remain mostly unclear.

Reactive oxygen species (ROS), which elicit oxidative stress causing a state of imbalance between the antioxidant defense system levels and the production of free radicals, are generated by environmental toxicants and reported to play a key role in cell signaling (such as cell proliferation and apoptosis) and to induce a wide variety of undesirable biological reactions that may lead to cell death and development of human diseases (including neurodegenerative diseases) (Jomova and Valko, 2011; Jomova et al., 2010). Oxidative stress destroys the homeostasis in pro-oxidant and antioxidant systems and has been reported to be an important mechanism in neurotoxicants-induced cell injuries (Chen et al., 2013; Lu et al., 2011a). Neuronal injuries have long been demonstrated to play a crucial role in toxic insults-induced oxidative stress leading to neuronal cells death and loss of brain functions, because the brain relies on aerobic respiration, consumes large quantities of oxygen, and has high poly-unsaturated lipid contents, making it particularly vulnerable to oxidative stress damages (Coyle and Puttfarcken, 1993; Loh et al., 2006). Some studies have indicated that the pathogenesis of neurodegenerative diseases (such as ischemic shock, Alzheimer's disease, and Parkinson's disease) is associated with the increase in ROS production causing neuronal cell death (Butterfield et al., 2002; Loh et al., 2006). Moreover, many recent studies have provided evidence that oxidative stress is significantly related to the development of toxic metals-induced pathophysiological progresses of neurodegenerative diseases, including arsenic (Ahmed et al., 2011; Jomova et al., 2011).

Increasing evidence indicates that toxic metals (such as mercury, copper, cadmium, and lead) can induce severe oxidative stress damage in mammalian cells that results in the destruction of DNA, proteins, and lipid functions as well as the development of many diseases (Chen et al., 2009; Jomova and Valko, 2011; Jomova et al., 2010). A significant relationship between neuronal cell death related to arsenic-induced ROS overproduction and the progression of neurodegenerative diseases, such as Alzheimer's disease, has been suggested (Bharathi et al., 2006; Gharibzadeh and Hoseini, 2008). Furthermore, arsenic-induced cytotoxic effects are closely associated with the production of oxidative stress damages and the activation of mitogen-activated protein kinases (MAPKs)/PI3K-Akt/mTOR/endoplasmic reticulum (ER) stress-regulated signaling pathways causing apoptosis in a variety of cell types, including leukemia, myoblasts, osteoblasts, and pancreatic β-cells (Estan et al., 2012; Lu et al., 2011b; Tang et al., 2009; Yen et al., 2012). However, the cytotoxic effects and mechanisms of arsenic in neuronal cells are still not understood. The aim of this study was to investigate the toxicological effects of arsenic in neuronal cells and to elucidate the cellular mechanism involved in arsenic-induced oxidative stress damage that caused neuronal cell apoptosis.

2. Materials and methods

2.1. Cell culture

Murine neuroblastoma cell line: Neuro-2a (CCL-131, American Type Culture Collection, Manassas, VA, USA) was cultured and maintained in plastic tissue culture dish in a humidified chamber with a 5% CO_2–95% air mixture at 37 °C and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (Gibco/Invitrogen, Carlsbad, CA, USA).

2.2. Determination of cell viability

Neuro-2a cells were seeded (2×10^4 cells/well) in 96-well plates and allowed to adhere and recover overnight. The cells were changed to fresh media and then