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Endoplasmic reticulum stress is involved in arsenite-induced oxidative injury in rat brain

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Abstract

The mechanism underlying sodium arsenite (arsenite)-induced neurotoxicity was investigated in rat brain. Arsenite was locally infused in the substantia nigra (SN) of anesthetized rat. Seven days after infusion, lipid peroxidation in the infused SN was elevated and dopamine level in the ipsilateral striatum was reduced in a concentration-dependent manner (0.3–5 nmol). Furthermore, local infusion of arsenite (5 nmol) decreased GSH content and increased expression of heat shock protein 70 and heme oxygenase-1 in the infused SN. Aggregation of α-synuclein, a putative pathological protein involved in several CNS neurodegenerative diseases, was elevated in the arsenite-infused SN. From the breakdown pattern of α-spectrin, both necrosis and apoptosis were involved in the arsenite-induced neurotoxicity. Pyknotic nuclei, cellular shrinkage and cytoplasmic disintegration, indicating necrosis, and TUNEL-positive cells and DNA ladder, indicating apoptosis was observed in the arsenite-infused SN. Arsenite-induced apoptosis was mediated via two different organelle pathways, mitochondria and endoplasmic reticulum (ER). For mitochondrial activation, cytosolic cytochrome *c* and caspase-3 levels were elevated in the arsenite-infused SN. In ER pathway, arsenite increased activating transcription factor-4, X-box binding protein 1, C/EBP homologues protein (CHOP) and cytosolic immunoglobulin binding protein levels. Moreover, arsenite reduced procaspase 12 levels, an ER-specific enzyme in the infused SN. Taken together, our study suggests that arsenite is capable of inducing oxidative injury in CNS. In addition to mitochondria, ER stress was involved in the arsenite-induced apoptosis. Arsenite-induced neurotoxicity clinically implies a pathophysiological role of arsenite in CNS neurodegeneration.

Keywords: Arsenite; Neurotoxicity; ER stress; α-Synuclein

Introduction

Due to its ubiquitous existence on the earth crust, arsenic can be found naturally in the ground water. A significant body of studies has focused on the health problems, including malignancies and cardiovascular diseases after consumption of a nonlethal level of arsenic in drinking water or food (Bernstam and Nriagu, 2000; Bolla-Wilson and Bleecker, 1987; Frank, 1976; Hall, 2002). Nowadays, daily intravenous infusion of arsenic trioxide is used in the clinic for the treatment of acute promyelocytic leukemia (Shen et al., 1997; Soignet et al., 1998). More and more human clinical trials explore the potential use of arsenic trioxide for the treatment of a variety of cancers (Kim et al., 2005; Lin et al., 2007). In these cases, over-exposure to arsenic is likely to induce unwanted side effects, including cardiotoxicity and neurotoxicity in patients treated with arsenic trioxide (Lin et al., 2007; Shen et al., 1997). For example, arsenic has been found to produce symmetric peripheral neuropathies, including numbness, paresthesiae of the distal extremities and sensorimotor loss (Bolla-Wilson and Bleecker, 1987; Frank, 1976; Lin et al., 2007; Rodriguez et al., 2003; Shen et al., 1997). Furthermore, systemic arsenic reportedly reduced levels of the biogenic amines in CNS (Tripathi et al., 1997) and induced CNS-related deficits, such as a reduction in locomotor activity and behavioral disorders (Itoh et al., 1990), encephalopathy, sleep disturbances, learning and memory deficits (Danan et al., 1984; Frank, 1976; Itoh et al., 1990).

Oxidative stress has been suggested as an underlying mechanism of arsenic-induced cytotoxicity (Bernstam and

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Nriagu, 2000). Over-production of free radicals, including nitric oxide (Chattopadhyay et al., 2002), peroxynitrite (Guidarelli et al., 2005), hydrogen peroxide (Chen et al., 1998) and hydroxyl radicals (Garcia-Chavez et al., 2003), has been demonstrated in arsenic-induced cytotoxicity. Ingestion of arsenic was found to reduce glutathione, superoxide dismutase and glutathione peroxidase in rat brain (Bashir et al., 2006; Flora, 1999; Garcia-Chavez et al., 2003; Shila et al., 2005a). Furthermore, activation of signal transduction pathways, such as JNK phosphatase, p38MAP kinases and Akt as well as modulation of transcription factors has been reported to be involved in arsenic-induced oxidative stress (Choi et al., 2002; Namgung and Xia, 2001). Taken together, arsenic may induce oxidative responses, forming oxidative DNA adducts (Bau et al., 2002) or attacking macromolecules including lipids and proteins (Shila et al., 2005b) which result in cell death.

α-Synuclein, the main component of inclusion bodies, is commonly found in several CNS neurodegenerative diseases, including Parkinsonism and Alzheimer's disease (Giasson et al., 2000; Vekrellis et al., 2004). One of the proposed mechanisms of α-synuclein resulting from a dysfunctional ubiquitin–proteasome system is to induce unfolding protein response (UPR) in endoplasmic reticulum and result in apoptosis (Inden et al., 2005). Indeed, several neurotoxins, including 1-methyl-4-phenylpyridinium (MPP^+) and 6-hydroxydopamine (6-OHDA), reportedly up-regulated α-synuclein expression and aggregation and caused damages in the dopaminergic neurons (Holtz and O'Malley, 2003; Inden et al., 2005) and neuroblastoma cells (Kalivendi et al., 2004). Due to the significant accumulation of arsenic in striatum (Itoh et al., 1990; Tripathi et al., 1997), the role of α-synuclein in arsenic-induced neurotoxicity in the nigrostriatal dopaminergic system needs to be determined.

A significant body of in vitro studies focused on arsenic-induced neuronal death (Chattopadhyay et al., 2002; Chen et al., 1998; Choi et al., 2002; Guidarelli et al., 2005; Mengesdorf et al., 2002; Namgung and Xia, 2001; Ramanathan et al., 2005; Scholz et al., 2005; Yang et al., 2003), and apoptosis is found to involve in arsenic-induced neurotoxicity in CNS (Bashir et al., 2006; Chattopadhyay et al., 2002; Flora, 1999; Mengesdorf et al., 2002; Ramanathan et al., 2005). The mitochondrial pathway has been reported to be involved in arsenic-induced apoptosis in neuronal cells (Chattopadhyay et al., 2002; Mengesdorf et al., 2002; Namgung and Xia, 2001; Ramanathan et al., 2005), however, the involvement of the ER pathway in the arsenic-induced neurotoxicity needs to be clarified. In the present study, the mechanism underlying sodium arsenite (arsenite)-induced neurotoxicity was studied in the nigrostriatal dopaminergic system of chloral hydrate-anesthetized rats. Oxidative stress was evaluated by measuring lipid peroxidation and GSH content in the arsenite-infused substantia nigra (SN). Furthermore, expression of α-synuclein and two stress proteins, heat shock protein 70 (HSP 70) and heme oxygenase-1 (HO-1), was monitored. Cleavage of α-spectrin was used to indicate the involvement of necrosis and/or apoptosis in arsenite-induced oxidative injury (Wang, 2000). The formation of 150 kDa/145 kDa fragments by calpain and 150 kDa/145 kDa/120 kDa fragments by caspases was employed to determine the involvement of necrosis and apoptosis, respectively (Wang, 2000). Moreover, the involvement of ER pathway in the arsenite-induced neurotoxicity was determined.

Materials and methods

Animals

Adult male Sprague–Dawley rats, weighing 250–350 g, were used. These animals were maintained according to the guidelines established in *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, USA (1985). The use of animals has been approved by the Institutional Animal Care and Use Committee of Taipei Veterans General Hospital, Taipei, Taiwan, R.O.C.

Chronic surgery and drug infusion

Sprague–Dawley rats were anesthetized with chloral hydrate (450 mg/kg, i.p., Sigma, St. Louis, MO) and placed in a stereotaxic instrument (David Kopf Instruments, Palo Alto, CA) with rectal temperature maintained at 37±1 °C. After skin incision and exposure of the parietal bone, holes were drilled above the cortical surface for intranigral infusion of drugs or solvent. Arsenite (0.3–5 nmol) in 1 μl filtered Ringer's solution (solvent) was infused stereotaxically (Paxinos and Watson, 1986) into SN (coordinates: 3.2 mm anterior and 2 mm above the interaural zero; 2.1 mm lateral to the midline; 3.5 mm below the incisor bar). Drug solutions or solvent were infused at a rate of 0.2 μl/min through a 30 gauge stainless steel needle. From the kinetics of the injected drug in the brain tissue, 10 s after microinjection, the diffusion areas for 10 nl extended to a radius of 225 μm where the concentration was reduced to 40% of the original concentration (Nicholson, 1985). The injection needle was held in place for an additional 3 min following drug infusion. At this time, the concentration of the injected substance at the injection site was decreased to less than 5% of the original concentration (Nicholson, 1985). After surgery, rats were allowed to recover from anesthesia and were placed in their home cages (from 1 h to 7 days) until sacrifice.

Fluorescence assay of lipid peroxidation in SN

At the end of each in vivo experiment, rats were sacrificed by decapitation. Intact SN (no any treatment) or SN infused with arsenite or solvent only was dissected and homogenized in chilled 400 μl chloroform and 200 μl methanol. After centrifugation, an aliquot of the chloroform and methanol layer was scanned using a spectrofluorometer (Aminco Bowman-2, USA). Lipid peroxidation expressed as relative fluorescence unit (RFU) was determined by measuring the levels of malondialdehyde and its dihydropyridine polymers, which emit fluorescence at 426 nm when activated by UV at 356 nm (Kikugawa et al., 1989).

HPLC-EC analysis of striatal dopamine content

Rats were decapitated and striata were dissected and immediately frozen in liquid nitrogen and stored at −70 °C until analysis. An HPLC (CC5/PM80, Bioanalytical Systems, Inc, IN, USA) with electrochemical detection (LC-4C, BAS, USA) procedure was used to quantify dopamine content in rat striatum. Applied potential was 0.75 volt vs Ag/AgCl. Mobile phase (liter) contained 2.1 g heptanesulfonic acid, 3.5 ml triethylamine, 3 ml phosphoric acid, 0.1 g NaEDTA and 170 ml acetonitrile. The retention time for dopamine was about 7.5 min (Chiueh et al., 1983).

Measurement of glutathione content in substantia nigra

GSH levels in SN were determined (Lin and Ho, 2000). In brief, frozen SN tissue was thawed in 5 volumes of 0.2 N perchloric acid and homogenized by sonication. Tissue homogenates were centrifuged at 12,000×*g* for 15 min.