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MiADMSA reverses impaired mitochondrial energy metabolism and neuronal apoptotic cell death after arsenic exposure in rats

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ABSTRACT

Arsenicosis, due to contaminated drinking water, is a serious health hazard in terms of morbidity and mortality. Arsenic induced free radicals generated are known to cause cellular apoptosis through mitochondrial driven pathway. In the present study, we investigated the effect of arsenic interactions with various complexes of the electron transport chain and attempted to evaluate if there was any complex preference of arsenic that could trigger apoptosis. We also evaluated if chelation with monoisoamyl dimercaptosuccinic acid (MiADMSA) could reverse these detrimental effects. Our results indicate that arsenic exposure induced free radical generation in rat neuronal cells, which diminished mitochondrial potential and enzyme activities of all the complexes of the electron transport chain. Moreover, these complexes showed differential responses towards arsenic. These early events along with diminished ATP levels could be co-related with the later events of cytosolic migration of cytochrome c, altered bax/bcl_2 ratio, and increased caspase 3 activity. Although MiADMSA could reverse most of these arsenic-induced altered variables to various extents, DNA damage remained unaffected. Our study for the first time demonstrates the differential effect of arsenic on the complexes leading to deficits in bioenergetics leading to apoptosis in rat brain. However, more in depth studies are warranted for better understanding of arsenic interactions with the mitochondria.

Introduction

Arsenic is a group V metalloid present ubiquitously in water, soil, and air. Arsenic is found in different chemical and oxidation states (−3, 0, +3, and +5) and causes acute and chronic adverse health effects (Hughes, 2002). Arsenic contaminated ground water is a major health problem in certain areas of the world especially parts of Bangladesh, United States, Taiwan, Mexico, Japan, and India. In addition, arsenic contamination in drinking water exceeds World Health Organization (WHO) provisional guideline value of 10 μg/l in many areas (Aposhian, 1998). Over 200 million people worldwide are at risk, out of which 112 million are residing in West Bengal, India (National Research Council (NRC), 2001), and Bangladesh, where groundwater arsenic concentrations exceed the WHO maximum permissible level of 50 μg/l (Steinmaus et al., 2005). Arsenic is known to interfere with a number of body functions including the central nervous system (Kannan et al., 2001; Flora et al., 2002; Smith et al., 1998).

Although the role of metals/metalloids in neurodegenerative diseases is controversial, recent reports suggest a significant co-relation between arsenic and lead with neuronal degeneration (Chattopadhyay et al., 2002; Flora et al., 2007b, Antonio et al., 1999). Data also indicate oxidative stress to be one of major causative factors for metal-induced neuropathies (Flora, 1999; Flora et al., 2007a,b; Figueiredo-Pereira et al., 1998) and may lead to malfunctioning of metabolic enzymes and ultimately apoptosis. This partial or complete loss of neurons might lead to disorders like Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). Bush (2003) correlated neuronal cell death in AD with oxidative stress particularly with excessive free radical generation. However, these metal-induced neuropathies are the major consequences of occupational hazards or environmental contaminations like high levels of metals in drinking water.

Several metal chelating agents have been tried and reported including British Anti-Lewisite (BAL), 2,3-dimercaptosuccinic acid (DMSA), and 2,3-dimercaptopropane sulfate (DMPS) for their effectiveness in treating heavy metal poisoning (Flora and Pachauri, 2010; Aposhian and Aposhian, 1990; Flora et al., 1995). However, most of them are known for their limitations and side effects that include low therapeutic index, non-specificity (essential metal loss such as copper and zinc), inability to penetrate cellular membrane (extracellular

Abbreviations: As (III), arsenic; ROS, reactive oxygen species; NO, nitric oxide; Mn-SOD, mitochondrial superoxide dismutase; $\Delta\Psi_m$, mitochondrial membrane potential (MMP); MiADMSA, monoisoamyl 2,3-dimercaptosuccinic acid; DCFDA, 2′,7′-dichlorofluorescein diacetate.

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nature), metal redistribution, hepatotoxicity, and renal toxicity (Mehta and Flora, 2001; Flora and Pachauri, 2010). We have previously demonstrated that mono-isoamyl ester of DMSA (MiADMSA) could efficiently remove heavy metals like arsenic and lead from acutely as well as chronically exposed animals (Mishra et al., 2008). It is now well known that arsenic induced oxidative stress leads to apoptosis via the mitochondrial pathway (Kitchin and Ahmad, 2003; Santra et al., 2007; Mishra et al., 2008; Flora et al., 2009).

However, no report currently demonstrates the effect of arsenic on the various complexes of the electron transport chain. In this study, we aimed to study the effect of arsenic on the various complexes of the electron transport chain and attempted to evaluate if there was any complex preference of arsenic that could trigger apoptosis. Furthermore, we also evaluated if chelation therapy with MiADMSA could reverse the detrimental effect of arsenic in sub-chronically exposed rat brain.

Methods

Chemicals and reagents. Sodium arsenite (98%; Sigma-Aldrich, USA) and all other chemicals were of analytical grade and were purchased from Merck (Germany), BDH Chemical (Mumbai, India), or Sigma (USA). Ultra pure water prepared by Millipore (New Delhi, India) was used throughout the experiment to avoid metal contamination and for the preparation of reagents/buffers used for various biochemical assays in our study. MiADMSA was synthesized in our synthetic chemistry division, by the controlled esterification of DMSA, with their corresponding alcohol in acidic medium (Jones et al., 1992). The product was purified (purity, 99.9%) and characterized using spectral and analytical methods before experimentation. The chemicals were stored in desiccators at 4 °C to avoid oxidation and thermal decomposition.

Animal studies. Male Wistar rats weighing 90 ± 10 g (~8 weeks old) were obtained from the animal house facility of Defence Research and Development Establishment (DRDE), Gwalior. The animal ethical committee of DRDE, Gwalior, India, approved the experimental protocol. The animals were housed in stainless steel cages in an air-conditioned room with temperature maintained at 25 ± 2 °C with 12 h alternating day and night cycles with relative humidity of 60%. Rats were allowed standard chow diet (Amrut Feeds; Pranav Agro, New Delhi; nutritional element content of diet, in parts per million dry weight: Cu, 10.0; Zn, 45.0; Mn, 55.0; Co, 5.0; Fe, 75.0) and water ad libitum throughout the study. Rats were randomly divided into 2 groups of 10 animals each and were treated 5 days a week for 4 weeks as follows:

Group I, normal animals ($n = 10$)
Group II, 2.5 mg/kg, arsenic as sodium arsenite, orally ($n = 10$)

After 4 weeks, animals in both the groups were further divided into 2 groups and treated as below for 5 consecutive days:

Group IA, normal animals ($n = 5$)
Group IB, MiADMSA, 50 mg/kg, orally, once, daily ($n = 5$)
Group IIA, arsenic (saline) ($n = 5$)
Group IIB, arsenic + MiADMSA, 50 mg/kg, orally, once, daily ($n = 5$)

Arsenic exposure was stopped during chelation therapy. The doses of arsenic and MiADMSA were selected based on our previous published reports (Flora et al., 2009; Flora et al., 2007b). Dose response evaluations of MiADMSA (25, 50, or 100 mg/kg) have shown 50 mg/kg to be the optimal dose for most effective recoveries in clinical variables as well as reducing arsenic body burden (Flora et al., 2003; Flora et al., 2007c). Based on these observations, we selected a dose of 50 mg/kg for this study. MiADMSA was dissolved in 5% sodium bicarbonate solution and solutions were prepared immediately before use. The dosing volume amounted to 4 ml/kg body weight. After completion of different treatments, animals were euthanized under light ether anaesthesia. Brains were removed, rinsed in chilled saline, blotted, weighed, and used for various assays.

Isolation of mitochondria. Rat brain mitochondria were isolated using a previously described method of Berman and Hastings (1999) modified by Brown et al. (2004). The brains were placed in glass Dounce homogenizer containing 5 volumes of isolation buffer with 1 mM ethylene glycol tetraacetic acid (EGTA), 215 mM mannitol, 75 mM sucrose, 0.1% BSA, 20 mM HEPES, and pH adjusted to 7.2 with KOH. Briefly, the tissue homogenate was spun at 1300*g* for 5 min in an Eppendorf microcentrifuge at 4 °C and the supernatant was transferred to new tubes. The loose pellet was resuspended in isolation buffer with EGTA and was spun again at 1300*g* for 3 min. The resulting supernatant was transferred to new microcentrifuge tubes and topped off with isolation buffer with EGTA and spun at 13,000*g* for 10 min in order to pellet the mitochondria. The supernatant was discarded and then the pellet was resuspended in 500 µl of isolation buffer with EGTA. Samples obtained using either method of cell disruption were brought up to a final volume of 2 ml with isolation buffer with EGTA and centrifuged at 13,000*g* for 10 min. The pellet was resuspended in isolation buffer without EGTA (215 mM mannitol, 75 mM sucrose, 0.1% BSA, 20 mM HEPES, and pH is adjusted to 7.2 with KOH) and centrifuged at 10,000*g* for 10 min. The final mitochondrial pellet was resuspended in isolation buffer without EGTA to yield a concentration of ~10 mg/ml and stored on ice. The integrity of mitochondria was checked by assessing respiratory control ratio (Han et al., 2003) (data not shown).

Cells from whole brain. Whole brain was isolated and washed with cold PBS with glucose and incubated with dispase (1 mg/ml) for 25–30 min followed by trypsin (0.05%) for 5–10 min. The clumps were gently dissociated with a serological pipette. The cells were then passed through a 0.70 µm filter to separate the clumps from the single cells. The cells were centrifuged at 1000*g* for 10 min. Cell pellet was resuspended in cold PBS and cells were counted using trypan blue. These cells were immediately used for various assays after preparation with or without different treatments (Pachauri et al., 2009).

NADH cytochrome c reductase (complexes I–III) activity. NADH cytochrome c reductase activity was measured spectrophotometrically by the method of King and Howard (1967). This method involves catalytic oxidation of NADH to NAD^+ with subsequent reduction of cytochrome c. The reaction mixture contained 0.2 M glycyl glycine buffer pH 8.5, 6 mM NADH in 2 mM glycyl glycine buffer, and 10.5 mM cytochrome c. The reaction was initiated by addition of requisite amount of solubilized mitochondrial sample and change in absorbance was followed at 550 nm for 2 min.

Succinate dehydrogenase (complex II) activity. Succinate dehydrogenase activity was measured spectrophotometrically as per the method of King (1967). This method involves oxidation of succinate by an artificial electron acceptor, potassium ferricyanide. The reaction mixture contained 0.2 M phosphate buffer pH 7.8, 1% BSA, 0.6 M succinic acid, and 0.03 M potassium ferricyanide. The reaction was initiated by the addition of mitochondrial sample and change in absorbance was followed at 420 nm for 2 min.

Cytochrome oxidase (complex IV) activity. Cytochrome oxidase activity was assayed in brain mitochondria according to the method of Sottocassa and Ernester (1967). The assay mixture contained 0.3 mM reduced cytochrome c in 75 mM phosphate buffer. The reaction was started by the addition of solubilized mitochondrial sample and change in absorbance was recorded at 550 nm for 2 min.