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Monoisoamyl 2,3-dimercaptosuccinic acid attenuates arsenic induced toxicity: Behavioral and neurochemical approach

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ABSTRACT

Chronic exposure to arsenic in drinking water is associated with skin lesions, neurological effects, hypertension and high risk of cancer. The treatment in use at present employs administration of thiol chelators, such as meso-2,3-dimercaptosuccinic acid (DMSA) which are compromised with number of limitations due to their lipophobic nature. To address this problem, therapeutic efficacy of monoisoamyl meso-2,3-dimercaptosuccinic acid (MiADMSA), an analog of DMSA having lipophilic character, was examined against chronic arsenic poisoning in rats. Adult male Wistar rats were orally exposed to arsenic (2 mg sodium arsenite/kg body weight) for 10 weeks followed by treatment with MiADMSA (50 mg/kg, orally, once daily for 5 consecutive days). As-exposed rats showed significant differences in behavioral functions (open field behavior, total locomotor activity, grip strength and exploratory behavior) and water maze learning. Further, the biochemical studies performed on three brain regions (cerebellum, cortex and hippocampus) also showed significant elevation in malondialdehyde (MDA) levels with a concomitant decrease in the oxidative stress marker enzymes Mn-superoxide dismutase (Mn-SOD), Cu/Zn-superoxide dismutase (Cu/Zn-SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST). The alterations were more pronounced in cortex compared to cerebellum and hippocampus. The results showed that MiADMSA significantly reversed the As-induced alterations in behavior and biochemical variables suggestive of oxidative injury.

1. Introduction

High concentration of naturally occurring arsenic in drinking water is a major health problem in different parts of the world (Ning et al., 2010). Exposure to arsenic at high levels can result in mild but persistent peripheral neuropathy (Kishi et al., 2001; Flora, 2011), sensory deficits (Hafeman et al., 2005; Otto et al., 2007) and impairment of higher neurological function (Rodriguez et al., 2003). In rodents, arsenic exposure is associated with altered neurotransmitter levels and operant learning deficits (Nagaraja and Desiraju, 1993, 1994) as well as lowered levels of spontaneous activity (Chattopadhyay et al., 2002a, 2002b; Rodriguez et al., 2001, 2002).

Arsenic exposure renders the brain tissue vulnerable to attack by free radicals resulting in abnormal apoptosis of

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neural cells. It is known that arsenic exposure induces overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body and results in nucleic acid damage to the nerve cells (Mishra and Flora, 2008; Flora, 2011). The exact mechanism of arsenic induced neurotoxicity, though unclear, has been partly attributed to oxidative injury (Rodriguez et al., 2002; Amrit et al., 2011; Dwivedi and Flora, 2011).

Although no general hypothesis is known for the mechanism to explain what cellular events underlie the behavioral and cognitive dysfunction in primates and non-primates, the detrimental effects of As have warranted interest in this area. The current approved clinical intervention method is to give chelating agents that form an insoluble complex with arsenic and remove it from arsenic-burdened tissues. The thiol and amino carboxylic acid metal chelators have been used for the prevention as well as therapy (Flora and Pachauri, 2010).

Monoesters of DMSA (meso-2,3-dimercaptosuccinic acid) were found to be highly efficient in the removal of cadmium (Kostial et al., 1994), mercury (Kostial et al., 1993) and arsenic from gallium arsenide exposed animals (Flora and Dwivedi, 2012). Significantly higher cadmium, mercury, lead, and arsenic mobilization in experimental animals has been reported after treatment with monoisoamyl meso-2,3-dimercaptosuccinic acid (MiADMSA), a C5-branched-chain alkyl monoester, than with DMSA (Gale et al., 1993; Blanusa et al., 1997; Pande et al., 2001; Flora et al., 2002a, 2002b; Flora and Pachauri, 2010). Recent studies have suggested that treatment with MiADMSA has no side-effects on blood, liver, and kidney biochemical variables following repeated administration in male and female rats (Mehta and Flora, 2001; Flora and Mehta, 2003).

In the present study we examined whether if treatment with MiADMSA during chronic arsenic intoxicated rats could be useful in functional recovery in altered behavioral activities.

2. Materials and methods

2.1. Chemicals

Sodium arsenite (purity 99.0%) was obtained from Sigma, USA, while MiADMSA (purity 99.9%) was a gift from Division of Regulatory Toxicology, DRDE, Gwalior, India.

2.2. Procurement and maintenance of experimental animals

Adult male albino rats (Wistar) of 3 months age were purchased from Sri Venkateswara Traders, Bangalore, and maintained in the animal house of Sri Venkateswara University, Dept. of Zoology. The animals were housed in clean plastic cages with hardwood bedding in a room maintained at $28 \pm 2\,^{\circ}C$ and relative humidity $60 \pm 10\%$ with a 12 h light/day cycle. The animals were fed with standard pellet diet supplied by Sri Venkateswara Traders, Bangalore, and, water ad libitum throughout the study. The protocol and animal use were approved by Institution Animal Ethical Committee, S.V. University. Rats were randomly divided into 2 groups and were treated 5 days a week for 10 consecutive weeks as follows:

Group I: Control animals (saline) ($n = 6$)
Group II: Arsenic as sodium arsenite (2 mg/kg, orally through gavage) ($n = 12$). After 10 weeks, animals in II group were further divided into 2 groups (IIA and IIB) and given the following treatment consecutively for 5 days:
Group IIA: Saline ($n = 6$)
Group IIB: MiADMSA, 50 mg/kg, orally.

The doses of arsenic and MiADMSA were selected on the basis of earlier published reports (Flora et al., 2007, 2009). 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 treatment, different behavioral activities were performed continuously for a period of 4 days and then the animals were sacrificed through cervical dislocation for estimating brain As levels and oxidative stress marker enzymes. Tissues were kept in ice-cooled conditions at all times.

2.3. Behavioral studies

2.3.1. Exploratory behavior

Exploratory behavior was evaluated in the hole board. The apparatus was an open-field arena with four equally spaced holes (3 cm in diameter) in the floor. Each rat was placed individually in the center of the arena for 5 min, during which we recorded head-dip count and head-dipping duration, in seconds. A head dip was scored if both eyes disappeared into the hole. Head-dipping duration data were expressed as total duration during the 5-min session. The results for head dip were expressed as number of counts, and for head-dipping duration in seconds.

2.3.2. Open-field behavior

The open field test has been widely used to assess emotional reactivity/anxiety. It provides measures of locomotor activity. The horizontally directed activity (or locomotion) is measured by the number of line crossings, and vertically directed activity (or exploration) is measured by the frequency of rearings (Prickaerts et al., 1998). The open-field behavior of rats was assessed in a wooden box measuring 90 cm × 90 cm × 30 cm high. The floor of the arena is divided into 36 equal squares by black lines. Immediately after a rat was placed in the center of the open field, the movement of the animal was scored. The number of squares crossed with all paws (crossings), the standings on the hind legs (rearings), placing the nose against wall or floor (sniffing), wiping, licking, combing or scratching of any part of the body (grooming) were counted in all sessions. All the activities measured were combined together to assess the mean total behavior in each session. Testing was carried out on five consecutive days in 5 min sessions in control, As-exposed and As + MiADMSA animals. The number of crossings indicates locomotor activity (Netto et al., 1986).

2.3.3. Locomotory activity

Total locomotor activity of the rat was studied with Opto-Varimex mini (Columbus Instruments, USA). The data was recorded as total activity for 30 min duration to each animal with 5 session intervals.