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Lipid oxidative damage and distribution of inorganic arsenic and its metabolites in the rat nervous system after arsenite exposure: Influence of alpha tocopherol supplementation

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Abstract

Inorganic arsenic (iAs) exposure causes peripheral neuropathy. Oxidative effects caused by iAs exposure in peripheral nerves have been incompletely characterized. This study analyzed arsenic and lipid oxidative damage in the brain, spinal cord, and sciatic and sensory sural nerves following arsenite exposure. This study also explored whether alpha tocopherol (α-TOC) administration mitigates arsenite-induced oxidative damage. Thiobarbituric acid-reactive substance (TBARS) levels and distributions of iAs and its metabolites were evaluated in male Wistar rats following 30 d of sodium arsenite exposure (10 mg/kg bodyweight (bw)/d, by gavage). A second group also received α-TOC (125 mg/kg bw/d, by gavage) during the final 20 d of arsenite administration. Arsenite exposure caused increased TBARS levels within each region of the nervous system; oxidative stress was most pronounced in the sural and sciatic nerves. In addition there was a positive quadratic relationship between TBARS levels and the concentration of arsenicals found in the nervous system ($r^2 = 0.878$, $p < 0.001$). Dimethylarsenic was the predominant metabolite of iAs found. Animals α-TOC-treated had a 1.7–5.2-fold reduction in TBARS levels when compared with rats that received iAs alone. These results suggest that oxidative damage may be the main mechanism of toxicity induced by exposure of the peripheral nervous system to arsenite and that such damage could be attenuated by α-TOC-supplementation.

Keywords: Arsenic; Neurotoxicity; Oxidative damage; Peripheral nervous system

1. Introduction

Inorganic arsenic (iAs) occurs naturally in the groundwater of many parts of the world and millions of people worldwide are exposed to drinking water containing this metalloid (ATSDR, 2000; NRC, 2001). Exposure to iAs causes many adverse human health effects, including cardiovascular, hepatic, renal and peripheral vasculature maladies, and dysfunction of the endocrine, nervous and reproductive systems, in addition to cancers of the skin, liver, lung, urinary bladder and kidney (NRC, 2001). To date there is limited information about the effects of iAs exposure upon the nervous system, particularly on the peripheral nervous system (PNS). Nevertheless arsenical exposure has been associated with paresthesia, weakness, and pain in the distal extremities (Mukherjee et al., 2003), to changes in sensory action potential propagation (Berdel-García et al., 2004; Hindmarsh et al., 1977; Le Quesne and McLeod, 1977; Oh, 1991; Yip et al., 2002) and axonal degeneration in the sural nerve (Goebel et al., 1990; Ota, 1970).

Metabolic conversion of iAs can significantly modify its toxic and cancer promoting effects (Thomas et al., 2004). Biomethylation is the major pathway for the metabolism of iAs. In humans, iAs is converted to methylarsenic (MAs) and dimethylarsenic (DMAs), which contain arsenic in a trivalent or pentavalent oxidation state (Thomas et al., 2004). In rats, hamsters and mice, DMAs is further methylated to yield trimethylarsine oxide (TMAO) (Devesa et al., 2004; Yoshida et al., 1998). Accumulation of iAs and its metabolites in the nervous system may facilitate the formation of reactive oxygen species (ROS) and thus lead to neurotoxicity.

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Under physiological conditions, the nervous system is especially susceptible to oxidative damage due to its high oxygen consumption rate, high levels of polyunsaturated fatty acids, and to its relatively low level of defense mechanisms against oxidant toxicity (Samson and Nelson, 2000). Oxidative stress has been implicated as an important element in many neuronal dysfunctions, affecting synaptic plasticity, dendritic morphology and neurogenesis (Zaidi and Banu, 2004).

Generation of ROS is the major mechanism by which iAs exerts its toxicity in several body tissues (Del Razo et al., 2001a). ROS have been demonstrated to be present in the rat brain exposed to iAs (Flora et al., 2005; García-Chávez et al., 2003). In addition, in rats iAs exposure decreases the concentration of brain glutathione (GSH) (Kannan and Flora, 2004) and reduce antioxidant enzyme activity, but increase the oxidant production (Shila et al., 2005a). The mechanism by which iAs affects the PNS has not been clearly established. The presence of methylated arsenic species in the nervous system could be responsible, at least in part, for the neurotoxic alterations reported, however the adverse effects of arsenical exposure have not yet been experimentally associated with methylated arsenic species. The potential role of oxidative stress in iAs neurotoxicity suggests that antioxidant supplementation may mitigate arsenite-induced toxicity.

Alpha tocopherol (α-TOC) is the most abundant and active form of vitamin E *in vivo* (Marcus and Coulston, 1994; Niki and Noguchi, 2004) and is an important lipophilic radical-scavenging antioxidant. It reacts with peroxyl radicals 10,000-fold faster than polyunsaturated lipids (Halliwell and Gutteridge, 2002). Therefore α-TOC is potentially useful as a therapeutic agent in the treatment of several disorders associated with oxidative damage. It could potentially be used to ameliorate the lipid peroxidation (LPO) induced by some pro-oxidants, including iAs. The proposed dose ranges for α-TOC therapeutic treatment vary widely. In humans, the reported therapeutic doses are between 200 and 3600 IU/d (Vatassery et al., 1999). In rats, the range has been between 10 and 1490 IU/kg body weight (bw)/d (Vatassery et al., 1999).

Although there has been great interest in the toxicological properties of iAs, there is a limited amount of information on the neurotoxicity of this metalloid. In this study, we compared LPO and tissue distribution of iAs and its metabolites in the central and PNS (brain, spinal cord and peripheral nerves) in rats exposed to arsenite. We selected two peripheral nerves: the sciatic and sural nerves, because both are easy to dissect from the rat limb. The sural nerve is predominantly sensory while the sciatic nerve is composed of sensory and motor axons.

2. Materials and methods

Sodium arsenite ($NaAs^{III}O_2$), dimethylarsinic acid [$DMAs^V$, $(CH_3)_2As^VO(OH)$], butylhydroxytoluene, deferoxamine mesylate, thiobarbituric acid, malondialdehyde bis-dimethylacetal, antifoam B silicone emulsion, and α-TOC were purchased from Sigma–Aldrich (St Louis, MO). Methylarsonic acid, disodium salt (MAs^V), $CH_3As^VO(ONa)_2$, was obtained from Ventron (Danvers, MA). Sodium borohydride ($NaBH_4$) and sodium hydroxide used in arsenic analysis were obtained from Merck (Mexico City). Ultrapure phosphoric acid was purchased from J.T. Baker (Phillipsburg, NJ). All chemicals used were of the highest commercially available purity. α-TOC solution was prepared in 96% ethanol and peanut oil (2:8, respectively, v/v), protected from light, and stored under a nitrogen atmosphere.

2.1. Laboratory animals and treatment

Male Wistar rats weighing 200–210 g of body weight (bw), were purchased from Cinvestav-IPN animal house. Rats received human care and the study complied with the Institution's guidelines and the Mexican Official Norm (NOM-062-ZOO-1999) regarding technical specifications for production, care, and use of laboratory animals. The animal room was kept on a 12/12-h light/dark cycle with the temperature set at 22 ± 1 °C and the humidity at 50 ± 5%. Animals were provided with standard rat chow (LabDiet® 5053 containing < 1 ppm arsenic, St. Louis, MO) and tap water containing < 4 μg iAs/l *ad libitum* throughout the experiment. All animals were allowed to acclimate to the facility for at least 2 weeks prior to treatment.

After a 15-d acclimatization period, rats (285–320 g bw) were randomly assigned to four experimental groups ($n = 8$ rats each), as summarized in Fig. 1: (A) *control rats*, which received 2.0 ml of deionized water/kg bw/d, by gavage, for 30 d; (B) *α-TOC rats*, which were supplemented with α-TOC, 125 mg/kg bw/d or 230 IU/kg bw/d, by gavage, for 20 d. As observed in previous reports, the α-TOC dose used in this study has no untoward effects and is considered a supplemental dose (Vatassery et al., 1999); (C) *sodium arsenic-treated rats* received a daily dose of 10 mg/kg bw of sodium arsenite dissolved in 2 ml of deionized water, by gavage, for 30 d. The dose of sodium arsenic used in this study was based upon previous reports in rodents which showed alterations in behavioral and locomotive activity after 15 d of daily exposure (Itoh et al., 1990; Rodriguez et al., 2001), and it represents 1/4

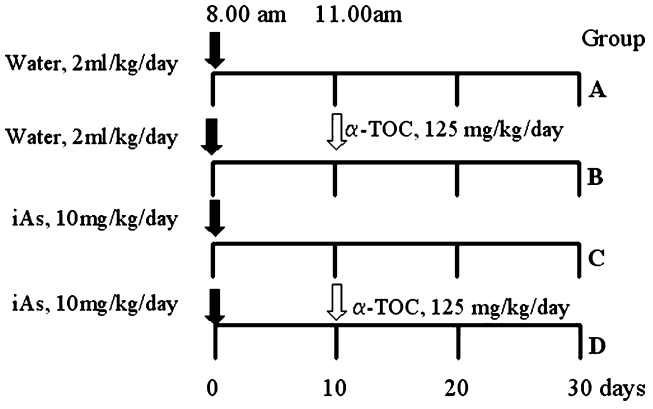

Fig. 1. Experimental design. Groups consisted of eight rats each: (A) control rats given deionized water only (2.0 ml/kg bw, by gavage, daily for 30 d); (B) control rats supplemented with α-TOC only (125 mg/kg bw, by gavage, daily on experimental days 10–30); (C) sodium arsenite-treated rats (10 mg/kg bw, by gavage, daily for 30 d); (D) sodium arsenite-treated rats with a similar dose of sodium arsenite as that of group C, plus α-TOC supplement at the same dose as group B, initiated 10 d after the administration of the first dose of arsenite.