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ScienceDirect
Toxicology and Applied Pharmacology 227 (2008) 26–35
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Tissue dosimetry, metabolism and excretion of pentavalent and trivalent dimethylated arsenic in mice after oral administration

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Received 18 July 2007; revised 5 October 2007; accepted 15 October 2007
Available online 22 October 2007

Abstract

Dimethylarsinic acid (DMA(V)) is a rat bladder carcinogen and the major urinary metabolite of administered inorganic arsenic in most mammals. This study examined the disposition of pentavalent and trivalent dimethylated arsenic in mice after acute oral administration. Adult female mice were administered [^{14}C]-DMA(V) (0.6 or 60 mg As/kg) and sacrificed serially over 24 h. Tissues and excreta were collected for analysis of radioactivity. Other mice were administered unlabeled DMA(V) (0.6 or 60 mg As/kg) or dimethylarsinous acid (DMA(III)) (0.6 mg As/kg) and sacrificed at 2 or 24 h. Tissues (2 h) and urine (24 h) were collected and analyzed for arsenicals. Absorption, distribution and excretion of [^{14}C]-DMA(V) were rapid, as radioactivity was detected in tissues and urine at 0.25 h. For low dose DMA(V) mice, there was a greater fractional absorption of DMA(V) and significantly greater tissue concentrations of radioactivity at several time points. Radioactivity distributed greatest to the liver (1–2% of dose) and declined to less than 0.05% in all tissues examined at 24 h. Urinary excretion of radioactivity was significantly greater in the 0.6 mg As/kg DMA(V) group. Conversely, fecal excretion of radioactivity was significantly greater in the high dose group. Urinary metabolites of DMA(V) included DMA(III), trimethylarsine oxide (TMAO), dimethylthioarsinic acid and trimethylarsine sulfide. Urinary metabolites of DMA(III) included TMAO, dimethylthioarsinic acid and trimethylarsine sulfide. DMA(V) was also excreted by DMA(III)-treated mice, showing its sensitivity to oxidation. TMAO was detected in tissues of the high dose DMA(V) group. The low acute toxicity of DMA(V) in the mouse appears to be due in part to its minimal retention and rapid elimination.
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Keywords: Arsenic; Dimethylarsinic acid; Dimethylarsinous acid; Trimethylarsine oxide; Thioarsenicals

Introduction

Arsenic is of concern to public health organizations because of its many different forms, its prevalence in the environment and exposure to it may result in adverse health outcomes. Exposure to the organic arsenical dimethylarsinic acid (DMA(V)) occurs from external and internal sources. Regarding external exposure, DMA(V) is found naturally in surface waters at levels ranging from 0.04 to 1 ppb (Braman and Foreback, 1973) and is used as a non-selective herbicide and a cotton desiccant (US EPA, 1975). Concerning internal exposure, DMA(V) is a major metabolite of inorganic arsenic (iAs) (Vahter, 1994) of most mammals as well as a metabolite of arsenosugars found in edible seaweed (Wei et al., 2003).

DMA(V) is readily absorbed by laboratory animals after oral administration (Buchet et al., 1981; Marafante et al., 1987; Stevens et al., 1977; Vahter et al., 1984; Yamauchi and Yamamura, 1984). The rat and mouse absorb from 65% to 85% of an oral dose of DMA(V) (Marafante et al., 1987; Stevens et al., 1977; Vahter et al., 1984), while the hamster absorbs less

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0041-008X/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.taap.2007.10.011

(ca. 50%) (Yamauchi and Yamamura, 1984). Humans also absorb DMA(V) following oral administration to volunteers (Buchet et al., 1981; Marafante et al., 1987) or occupational exposure (Tarrant and Allard, 1972; Wagner and Weswig, 1974). Absorbed DMA(V) is excreted rapidly in urine by most mammals (Hughes and Kenyon, 1998; Marafante et al., 1987; Vahter et al., 1984). However, the rat is unique, as it avidly retains DMA(V) (Stevens et al., 1977; Vahter et al., 1984), resulting in an unusually long whole-body half life of this arsenical. DMA(V) is reduced *in vivo* to dimethylarsinous acid (DMA(III)), which the rat red blood cell effectively takes up (Shiobara et al., 2001). DMA(III) binds specifically to cysteine-13-alpha of rat hemoglobin, more strongly than other arsenic compounds (Lu et al., 2004, 2007).

In vivo laboratory studies conducted 15–20 years ago report that DMA(V) is excreted in urine primarily unchanged after oral and parenteral administration (Marafante et al., 1987; Vahter et al., 1984; Yamauchi and Yamamura, 1984). It was observed that 4–15% of the dose of DMA(V) administered orally was excreted as a trimethylarsenic compound in urine of the hamster (Marafante et al., 1987; Yamauchi and Yamamura, 1984), mouse (Marafante et al., 1987), rat (Chen et al., 1996; Yoshida et al., 1997, 1998) and man (Marafante et al., 1987). This compound has been identified as trimethylarsine oxide (TMAO) (Marafante et al., 1987; Yoshida et al., 1997). Current studies with improved analytical techniques have detected DMA(III) and dimethylthioarsenicals in tissues and urine of animals exposed to DMA(V) (Cohen et al., 2002; Lu et al., 2003; Suzuki et al., 2004a; Yoshida et al., 2003). There are reports that DMA(V) is demethylated *in vivo* to monomethylarsonic acid (MMA(V)) and inorganic arsenic (iAs), products which were detected in the urine of DMA(V)-treated rats (Chen et al., 1996; Yamauchi and Yamamura, 1984; Yoshida et al., 1997). However, demethylated products of administered DMA (V) have not been reported by other investigators using rats, mice, hamsters or human volunteers (Buchet et al., 1981; Hughes et al., 2000; Marafante et al., 1987; Stevens et al., 1977; Vahter et al., 1984).

The acute toxic effects of DMA(V) are minimal (Kenyon and Hughes, 2001); the oral LD_{50} of DMA(V) ranges from 644 to 1800 mg/kg in the rat and mouse (Gaines and Linder, 1986; Kaise et al., 1989). The acute *in vivo* effects of DMA(III) are not known, but it is more highly cytotoxic than DMA(V) (Cohen et al., 2002; Dopp et al., 2004; Styblo et al., 2000). In contrast to its relatively low acute toxic potency, DMA(V) is a multi-organ tumor promoter in mice (Yamanaka et al., 1996, 2000) and rats (Li et al., 1998; Wanibuchi et al., 1996; Yamamoto et al., 1995) and a complete carcinogen for rat urinary bladder following chronic exposure in drinking water (50–200 ppm) (Wei et al., 1999, 2002) or the diet (100 ppm) (Arnold et al., 2006). Knowledge of the metabolic fate of arsenic derived from orally administered DMA(V) will facilitate evaluation of the role of DMA(V) and its metabolites in arsenic-induced toxicity. The objective of the present study was to examine the dosage dependency on the tissue dosimetry, metabolism and excretion of arsenicals following the oral administration of DMA(V) or DMA(III) in the mouse.

Methods

Chemicals. [^{14}C]-Dimethylarsinic acid (specific activity, 10 mCi/mmol) was purchased from ICN Radiochemicals (Irvine, CA, USA). The radiochemical purity of the compound was greater than 98% (Hughes and Thompson, 1996). Unlabeled DMA(V) (90% purity, 9% MMA(V), 1% iAs) was from Ansul (Weslaco, TX, USA). Dimethylarsine iodide (purity, 98%) and TMAO (purity >98%) were synthesized by Dr. William Cullen (University of British Columbia, Vancouver, British Columbia, Canada). Carbo-sorb E, Permafluor E and Ultima Gold were obtained from Perkin Elmer (Meriden, CT, USA). Sodium borohydride, sodium hydroxide and ACS certified hydrochloric acid were purchased from EM Science (Gibbstown, NJ, USA). Antifoam B silicone emulsion and phosphoric acid (Ultrex grade) were obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). All other chemicals used were of the highest grade commercially available.

Animals. Female B6C3F1 mice were obtained from Charles River Laboratories (Raleigh, NC, USA). The mice were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care and maintained according to the guidelines in the National Institutes of Health *Guide on the Care and Use of Laboratory Animals*. The animals were initially group housed in polycarbonate cages with bedding of heat-treated pine shavings. The mice were provided Prolab RMH300 (Purina, St. Louis, MO, USA) (containing <1 ppm As) and tap water *ad libitum* throughout the experiment. The animal room was on a 12/12-h light/dark cycle and the temperature and humidity were 22±1 °C and 50±10%, respectively. The mice were moved to metabolism cages (Nalge Co., Rochester, NY, USA) 3 days before the experiment began. On the day of dosing, the average weight of the animals was 25 g and they ranged in age from 90 to 120 days.

Experimental. In the dosimetry study, mice were administered [^{14}C]-DMA (V) (2 μCi) at dosage levels of 0.6 and 60 mg As/kg body weight. These dosages correspond to 1.11 and 111 mg/kg of DMA(V), respectively. The dosing solutions were prepared in HPLC-grade water (Burdick and Jackson, Muskegon, MI) and administered orally (10 ml/kg). The mice were housed in individual metabolism cages after dosing. At selected time points (0.25, 0.5, 1, 2, 4, 8, 12 or 24 h), four mice at each dosage level of DMA(V) were sacrificed by cardiac puncture under CO_2-induced anesthesia. Kidney, liver, lung and urinary bladder were removed from the mice. The blood and organs were weighed, frozen in liquid nitrogen and stored at −70 °C until processed for radioassay by combustion in a Perkin Elmer model D307 oxidizer. Urine and feces were collected from each metabolism cage at the time of sacrifice. Each metabolism cage was washed with approximately 75 ml of 10% (v/v) Count-Off (Perkin Elmer). This wash was mixed with the urine and brought to a total volume of 100 ml. Aliquots of the combined urine and cage wash were mixed with scintillant and radioassayed in a Perkin Elmer model 2560 liquid scintillation counter. The feces were weighed and combusted before radioassay.

In the metabolism study, mice were administered unlabeled DMA(V) (0.6 or 60 mg As/kg) or DMA(III) (0.6 mg As/kg) by oral gavage (10 ml/kg). Three mice for each dosage group were housed individually in metabolism cages and urine was collected cold for 24 h. Three control mice were administered water. Urine samples were analyzed for speciated arsenic on the day of collection by hydride generation-atomic absorption spectroscopy (HG-AAS) as described below. Other 24-h urine samples were frozen at −70 °C until analyzed by mass spectrometry for methylated thioarsenic compounds as described below. Another group of mice (N=3/group) were similarly treated but sacrificed 2 h post-administration of DMA(V), DMA(III) or water. Blood, kidney, liver, lung and urinary bladder were removed from these mice, weighed, frozen in liquid nitrogen and stored at −70 °C. At a later time, these tissues were digested in ultra pure phosphoric acid and analyzed by HG-AAS as described below.

Atomic absorption spectrometry of arsenicals. Urine and acid digested tissues of mice treated with DMA(V), DMA(III) or water were analyzed by HG-AAS as described by Devesa et al. (2004) with some modifications based on Hernandez-Zavala et al. (in press). Briefly, arsines are generated from arsenicals within the samples using sodium borohydride. The arsines are flushed into a U-tube, which is packed with 15% OV-3 (coated C-18) (Supelco, Inc., Bellefonte, PA, USA). Arsines are cryotrapped onto the chromatography packing by immersing the U-