Available online at www.sciencedirect.com

SCIENCE DIRECT®

Toxicology and Applied Pharmacology 208 (2005) 186–197

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

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

Michael F. Hughes[a,*], Vicenta Devesa[b], Blakely M. Adair[a], Miroslav Styblo[c], Elaina M. Kenyon[a], David J. Thomas[a]

[a]*U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC 27711, USA*

[b]*University of North Carolina at Chapel Hill, Center for Environmental Medicine, Asthma and Lung Biology, Chapel Hill, NC 27599, USA*

[c]*University of North Carolina at Chapel Hill, Department of Pediatrics, School of Medicine, Chapel Hill, NC 27599, USA*

Received 3 December 2004; accepted 15 February 2005
Available online 5 April 2005

Abstract

Exposure to monomethylarsonic acid (MMA(V)) and monomethylarsonous acid (MMA(III)) can result from their formation as metabolites of inorganic arsenic and by the use of the sodium salts of MMA(V) as herbicides. This study compared the disposition of MMA(V) and MMA(III) in adult female B6C3F1 mice. Mice were gavaged po with MMA(V), either unlabeled or labeled with ^{14}C at two dose levels (0.4 or 40 mg As/kg). Other mice were dosed po with unlabeled MMA(III) at one dose level (0.4 mg As/kg). Mice were housed in metabolism cages for collection of excreta and sacrificed serially over 24 h for collection of tissues. MMA(V)-derived radioactivity was rapidly absorbed, distributed and excreted. By 8 h post-exposure, 80% of both doses of MMA(V) were eliminated in urine and feces. Absorption of MMA(V) was dose dependent; that is, there was less than a 100-fold difference between the two dose levels in the area under the curves for the concentration-time profiles of arsenic in blood and major organs. In addition, urinary excretion of MMA(V)-derived radioactivity in the low dose group was significantly greater ($P < 0.05$) than in the high dose group. Conversely, fecal excretion of MMA(V)-derived radioactivity was significantly greater ($P < 0.05$) in the high dose group than in the low dose group. Speciation of arsenic by hydride generation-atomic absorption spectrometry in urine and tissues of mice administered MMA(V) or MMA(III) found that methylation of MMA(V) was limited while the methylation of MMA(III) was extensive. Less than 10% of the dose excreted in urine of MMA(V)-treated mice was in the form of methylated products, whereas it was greater than 90% for MMA(III)-treated mice. In MMA(V)-treated mice, 25% or less of the tissue arsenic was in the form of dimethylarsenic, whereas in MMA(III)-treated mice, 75% or more of the tissue arsenic was in the form of dimethylarsenic. Based on urinary analysis, administered dose of MMA(V) did not affect the level of its metabolites excreted. In the tested range, dose affects the absorption, distribution and route of excretion of MMA(V) but not its metabolism.

Keywords: Arsenic; Monomethylarsenic; Dosimetry; Metabolism

Introduction

The metalloid arsenic occurs in a variety of inorganic and organic forms. The multiplicity of arsenicals, each with unique chemical and toxicological activities, complicates the risk management for human exposure to this diverse group of compounds. Monomethylarsonic acid (MMA(V)), an organic arsenical in the pentavalent oxidation state, is of public health significance because its mono- and disodium salt forms are registered herbicides in the United States. In

Abbreviations: MMA(V), monomethylarsonic acid; MMA(III), monomethylarsonous acid; DMA(V), dimethylarsinic acid; DMA(III), dimethylarsinous acid; MSMA, monosodium methanarsonate; MMA, monomethylarsenic(III + V); DMA, dimethylarsenic(III + V); TMAO, trimethylarsine oxide; HG, hydride generation; AAS, atomic absorption spectrometry; AFS, atomic fluorescence spectrometry; AUC, area under the curve; iAs, inorganic arsenic; CL, clearance; F, relative bioavailability; GST-O, glutathione-*S*-transferase omega.

* Corresponding author.
E-mail address: hughes.michaelf@epa.gov (M.F. Hughes).

0041-008X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2005.02.008

addition, MMA(V) is a methylated metabolite found in the urine of humans exposed to inorganic arsenic (iAs) (Aposhian et al., 2000; Del Razo et al., 2001; Mandal et al., 2001). Hence, exposure to MMA(V) can occur externally by its use as a pesticide and internally from the metabolism of iAs.

MMA(V) administered po is well absorbed by several species, including humans. Based on urinary excretion data, 75% or more of a dose of MMA(V) administered po was absorbed by goats (10 mg As/kg), sheep (10 mg As/kg) (Shariatpanahi and Anderson, 1984) and humans (500 μg As) (Buchet et al., 1981). The absorbed dose was excreted in urine fairly rapidly by these species. By comparison, hamsters appear to absorb less MMA(V) than other species. By 24 h post-dosing, 56% of a po dose (27 mg As/kg) of MMA(V) was eliminated in feces (Yamauchi et al., 1988). In contrast, biliary excretion of MMA(V) administered ip (27 mg As/kg) to hamsters was minimal, as 78% of the administered dose was excreted in urine by 24 h (Yamauchi et al., 1988).

MMA(V) is not extensively metabolized by mammals to methylated products. Less than 10% of a po administered dose of MMA(V) to humans (500 μg As) (Buchet et al., 1981) or hamsters (3–166 mg As/kg) (Yamauchi et al., 1988) or iv to mice (0.4 or 40 mg As/kg) (Hughes and Kenyon, 1998) or rats (0.2–0.25 mg/kg) (Cui et al., 2004; Suzuki et al., 2004a) was converted to dimethylarsenic species. In vitro studies with rat liver cytosol (Styblo et al., 1995), rat and human hepatocytes (Styblo et al., 1999) or human recombinant arsenic methyltransferase (Hayakawa et al., in press) also show that methylation of MMA(V) was minimal. In contrast, monomethylarsonous acid (MMA(III)) (trivalent monomethylarsenic) was readily methylated in vitro to dimethylarsenic (Styblo et al., 1995, 1999). Recent studies with trivalent monomethylarsenic, formed by reacting MMA(V) with thiols, and administered po (0.1–0.5 mg/kg) (Cui et al., 2004) or iv (0.5 mg/kg) (Suzuki et al., 2004b) to rats showed that it was methylated to DMA(V). The low rate of conversion of MMA(V) is surprising, given the relative rapidity of iAs methylation by many species. Based on a proposal by Challenger (1945), metabolism of arsenic involves sequential reduction and oxidative methylation. MMA(V) is an intermediary in this pathway to the primary arsenical metabolite, dimethylarsinic acid (DMA(V)). However, Hayakawa et al. (in press) recently proposed that MMA(V) is not an intermediary of iAs methylation, but a product of the intermediate monomethylarsenic diglutathione. Regardless of the pathway of iAs metabolism, both MMA(V) and MMA(III) are excreted in the urine of individuals that consume drinking water contaminated with iAs (Aposhian et al., 2000; Del Razo et al., 2001; Le et al., 2000; Mandal et al., 2001).

MMA(V) is relatively non-toxic after acute exposure. The oral LD50s of MMA(V) and its sodium salts are 1.8 g/kg in mice (Kaise et al., 1989) and >800 mg/kg (Gaines and Linder, 1986) in rats. The reproductive capacity of male mice repeatedly administered the monosodium salt of MMA(V) (MSMA) (11.9 and 119 mg/kg) over several weeks was reduced (Prukop and Savage, 1986). Hepatic inflammation was observed in rabbits after dietary exposure to MSMA (50 ppm) for 7 or 12 weeks (Exon and Harr, 1974). MMA(V) does not appear to be a carcinogen. In a 2-year MMA(V) dietary study, tumors were not observed in mice (10–400 ppm) or rats (50–130 ppm) (Arnold et al., 2003). The large intestine was the primary target organ of MMA(V)-induced toxicity for both species, producing non-tumorigenic lesions in the rectum, colon and cecum.

In contrast to MMA(V), MMA(III) is a potent acute toxicant. In hamsters, the LD50 of MMA(III) after ip administration (29.3 μmol/kg) was 3–4 times lower than the LD50 of arsenite (112 μmol/kg) (Petrick et al., 2001). MMA(III) was a more potent cytotoxicant than arsenite in human Chang liver cells (Petrick et al., 2000), human hepatocytes (Styblo et al., 2000) and several other human and rodent cell types (Styblo et al., 2000). MMA(III) was a direct-acting genotoxin, whereas MMA(V) was not active (Andrewes et al., 2003; Kligerman et al., 2003; Mass et al., 2001). The chronic effects of exposure to MMA(III) are not known. Because of the differences in toxicity between MMA(V) and MMA(III), the hypothesis that methylation of iAs is a detoxication mechanism has been questioned. The formation of methylated metabolites of iAs that retain trivalent arsenic is a mechanism for the activation of arsenic to reactive and toxic species.

This study examined the effect of dose on the tissue dosimetry, metabolism and excretion of MMA(V) after po administration to female B6C3F mice. These results were also compared to those of a single dose of MMA(III).

Methods

Chemicals. [^{14}C]-Disodium monomethylarsonate (specific activity, 10 mCi/mmol) was obtained from ICN Radiochemicals (Irvine, CA, USA). The radiochemical purity of the compound, determined by ion chromatography/radioflow detection (Hughes and Thompson, 1996), was greater than 98%. DMA(V) was purchased from Ansul (Weslaco, TX, USA). Sodium arsenate and sodium arsenite were obtained from Sigma (St. Louis, MO, USA). The disodium salt of MMA(V) (purity, 99%) was purchased from AccuStandard (New Haven, CT, USA). Monomethylarsine oxide (MMA(III)) (purity, 98%) and dimethylarsine iodide (DMA(III)) (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 nitric, sulfuric, perchloric and hydrochloric acids used in the total arsenic analysis were purchased from EM Science (Gibbstown, NJ, USA). Antifoam B silicone emulsion and phosphoric acid (Ultrapure) were from