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

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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 ¹⁴C 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

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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 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 $\mu\text{mol/kg}$) was 3–4 times lower than the LD50 of arsenite (112 $\mu\text{mol/kg}$) (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/radio-flow 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 Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Freeze-dried urine standard reference materials for toxic metals (SRM 2670, National Institute of Standards and Technology, Gaithersburg, MD, USA) containing a low (60 ng/ml) and high level (480 ng/ml) of arsenic were used for total arsenic analysis. All other chemicals used were of the highest grade commercially available.

Animals

Female B6C3F1 mice were from Charles River Laboratories (Raleigh, NC, USA). Mice were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care and maintained in accordance with the *Guide on the Care and Use of Laboratory Animals*. Mice were initially group housed in polycarbonate cages with bedding of pine shavings and were provided Prolab RMH300 (Purina, St. Louis, MO, USA) (containing <1 ppm arsenic) and tap water ad libitum throughout the experiment. Mice were transferred to metabolism cages (Nalge Co., Rochester, NY, USA) 5 days before dosing. The animal room was on a 12:12-h light/dark cycle, the temperature was $22 \pm 1^\circ\text{C}$ and humidity was $50 \pm 10\%$. The average weight of the treated mice was 25 gm and their age ranged from 90 to 120 days.

Experimental

The [^{14}C]-MMA(V) dosing solution was prepared in HPLC-grade water (Burdick and Jackson, Muskegon, MI, USA) and administered by po gavage (10 ml/kg) at a dose level of 0.4 or 40 mg of elemental As per kg body weight. These doses correspond to 4.8 and 480 $\mu\text{mol/kg}$ of

MMA(V) (disodium salt) (1.4 and 140 mg/kg), respectively. Each animal was administered approximately 1.2 μ Ci of radioactivity. Mice were housed individually in metabolism cages after dosing. Mice were sacrificed by exsanguination (cardiac puncture) under CO₂-induced anesthesia at 0.25, 0.5, 1, 2, 4, 8, 12 or 24 h post-dosing. Unless otherwise indicated, there were four mice/time point. Kidney, liver, lung and urinary bladder were removed from the mice. Tissues were weighed, frozen in liquid nitrogen and stored at -70 °C until processed for analysis of radioactivity. Processed samples were combusted in a Perkin Elmer model D307 oxidizer. Radioactivity was analyzed in a Perkin Elmer 2560 liquid scintillation counter. Urine and feces were collected at the time of sacrifice. Each metabolism cage was washed with 75 ml of diluted Count-Off (DuPont, Boston, MA, USA). The cage wash was collected and diluted to 100 ml with distilled water. Aliquots of urine and cage wash were mixed with scintillant and assayed for radioactivity. The urine and cage wash radioactivity data were combined. Feces were weighed and combusted before analysis for radioactivity.

In metabolism studies, mice were housed individually in metabolism cages and administered po unlabeled MMA(III) (0.4 mg As/kg, 0.56 mg MMA(III)/kg) or MMA(V) (0.4 or 40 mg As/kg). Control mice were administered water. Urine was collected for 24 h on dry ice. Mice were sacrificed as described above and tissues (urinary bladder, kidney, liver and lung) were removed. Urine of control and MMA(III)-treated mice was analyzed for speciated arsenicals (see below) on the day of collection, while urine of MMA(V)-treated mice was stored at -70 °C until similarly analyzed. Total arsenic was determined in urine collected from all treatment groups that was stored at -70 °C. A second group of mice were sacrificed 2 h after administration of MMA(III) or MMA(V). Tissues were removed, weighed, frozen in liquid nitrogen and stored at -70 °C until analyzed for arsenicals as described below. Results from the tissue time course study indicated that the peak tissue concentration of MMA(V)-derived radioactivity was between 0.25 and 4 h. Therefore, the 2-h time point was chosen to collect and analyze tissues for speciated and total arsenic.

Speciation of aliquots of cumulative 24-h urinary arsenic was done by pH selective hydride generation (HG)-atomic absorption spectrometry (AAS) as described by Devesa et al. (2004). This method used a Perkin-Elmer model 5400 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA) coupled with a manual hydride generation system at pH 1 or pH 6 and a glass U-tube packed with Chromosorb WAW-dimethyldichlorosilane 46/60 (Supelco, Inc., Bellefonte, PA) immersed in a liquid nitrogen cold trap. With this methodology, arsines from trivalent arsenicals and trimethylarsine oxide (TMAO) are selectively generated at pH 6, cold-trapped and separated by their boiling points. At pH 1, arsines are generated from both tri- and pentavalent arsenicals. Detection limits ranged from 0.15 ng for TMAO to 0.4 ng for iAs (Devesa et al., 2004).

Tissue samples were digested before HG-AAS analysis. Liver, kidney and lung samples were homogenized in deionized water (100 mg tissue/ml water). Urinary bladder was digested without homogenization. Samples were digested in 3 ml of 2 M ultrapure phosphoric acid at 90 °C overnight. For low dose samples, 0.25 ml of homogenate (25 mg of tissue) was digested. For high dose samples, 0.1 ml of homogenate (10 mg of tissue) was digested. For blood, 0.25 ml aliquots were digested. Tissue samples were analyzed by the method as described above for urine. However, the analysis was carried out only at pH 1 because complete oxidation of trivalent arsenicals to pentavalency occurs during digestion. Thus, the oxidation state of monomethyl (MMA)- and dimethylarsenic (DMA) detected in tissues cannot be distinguished by this method. Stability of the arsenicals during digestion was tested by incubating aqueous standards in phosphoric acid overnight at 90 °C. Recoveries of speciated arsenicals were tested by analysis of total arsenic contents by HG-atomic fluorescence spectrometry (AFS) as described below.

Identification and quantification of arsenic in urine and tissues were performed by spiking samples with standards at different concentrations. Calibration curves for 0.5, 2.5, 10, 20 and 80 ng As for each standard (pH 1, sodium arsenate, MMA(V), DMA(V); pH 6, sodium arsenite, MMA(III), DMA(III), TMAO) were generated to quantify results for the speciation analyses at both pH 1 and 6. Stock solutions of 100 ng/ml of As were used for calibration. The peak area was used to quantify determinations made in urine and tissues. It should be noted that no standard reference material certified for analysis of mono-, di- and trimethylated arsenicals is currently available. Therefore, sample aliquots spiked with authentic As standards were used for quality control during the speciation analysis of As.

Total arsenic in mouse urine and tissues was analyzed by HG-AFS using an automated ProStar 410 autosampler and 9012 quaternary pump (Varian Analytical Instruments, Palo Alto, CA, USA) with a Millennium Excalibur HG-AFS system (PS Analytical Ltd., Kent, England). Urine and tissues were completely wet-digested by sequential addition of nitric, sulfuric and perchloric acids as described by Cox (1980) and modified by Devesa et al. (2004). This digestion converts all arsenicals to iAs for HG-AFS determination. The instrumental limit of detection was 0.15 ng As. Recovery of arsenic from urine and tissues was calculated by comparing the sum of the concentrations of arsenic species detected by HG-AAS with total arsenic concentration values determined by HG-AFS ($((\sum[\text{arsenic species}]) / [\text{total arsenic}]) \times 100$).

Data are listed throughout the text as mean $\pm$ SD. SAS System for Windows (Version 8.2; Cary, NC, USA) was used to analyze the data. Urinary and fecal excretion data were analyzed by PROC GLM with repeated measures and classifications of dose and time. Significant dose effects were further tested at each time point using Fisher's least significant-difference test. Tissue data were analyzed by PROC GLM and classifications of dose and time. Significant dose effects were further tested at each time point by Fisher's least significant-difference test. Effects were considered statistically significant at $P < 0.05$. PK Solutions Version 2.06 (Summit Research Services, Montrose, CO, USA) was used to determine the area under the curve (AUC) for the arsenic tissue concentration-time profiles. The relative bioavailability (F) for each dose of administered MMA(V) was determined by the following equation: $F = (X_{po}/X_{iv})$, where X represents the 24-h cumulative amount of MMA(V)-derived radioactivity excreted in urine after po or iv administration. The iv data were from Hughes and Kenyon (1998). Clearance (CL) of MMA(V)-derived radioactivity from the blood was determined by the following equation: $CL = (\text{dose} * F) / AUC_{\text{blood}}$.

Results

MMA(V) was rapidly absorbed and distributed throughout the mouse after po administration; MMA(V)-derived radioactivity was detected at the earliest time point, 0.25 h post-dosing, in blood and all organs examined. Peak tissue concentrations of MMA(V)-derived radioactivity, based on % dose/g, occurred between 0.25 and 4 h post-administration for both dose groups (Fig. 1). Peak concentrations of MMA(V)-derived radioactivity were greater in bladder, kidney and lung than in blood, suggesting uptake of arsenic by these tissues. Urinary bladder from 0.4 mg As/ kg MMA(V)-dosed mice, 4 h post-exposure, had the highest concentration ($1.47 \pm 0.82\%$) of the organs analyzed. Other than 15 min post-exposure for liver of the 0.4 mg As/kg dose group (liver: $0.45 \pm 0.37\%$; blood: $0.22 \pm 0.04\%$), the similar levels of MMA(V)-derived radioactivity between blood and liver suggested limited hepatic uptake of MMA(V). Significant effects of time on the concentration of MMA(V)-derived radioactivity were found in all tissues. Significant effects of dose and the interaction of dose $\times$ time on the concentration of MMA(V)-derived radioactivity were observed in blood, liver and lung. Significant differences in the concentration of MMA(V)-derived radioactivity were detected at several time points in blood, liver and lung (Fig. 1).

Tissue concentration-time profiles, based on ng As/g tissue (Fig. 2), appeared parallel for both doses of MMA(V). Comparison of the area under the curves (AUC) of MMA(V)-derived radioactivity in blood and organs showed there was a less than a 100-fold difference between the two administered doses (Table 1), which is suggestive of dose-dependent effects in disposition of MMA(V).

The terminal or elimination half-lives of MMA(V)-derived radioactivity in tissues of 0.4 mg As/kg dosed mice ranged from 4 h in liver to 15 h in kidney (Table 1). In tissues of 40 mg As/kg dosed mice, the elimination half-lives ranged from 4 h in liver to 13 h in kidney.

MMA(V)-derived radioactivity was rapidly excreted in urine and feces after po administration (Fig. 3). Mice administered 0.4 or 40 mg As/kg [^{14}C]-MMA(V) excreted approximately 80% of the radiolabel by 8 h after treatment in urine and feces. Statistical analysis of the urinary and fecal data showed significant effects of dose and time. For urine, significantly greater excretion of the 0.4 mg As/kg dose occurred at 0.25, 4, 8, 12 and 24 h. For feces, significantly greater excretion of the 40 mg As/kg dose occurred at 8, 12 and 24 h.

The relative bioavailability of MMA(V)-derived radioactivity was 0.81 and 0.6 for 0.4 and 40 mg As/kg MMA(V), respectively. Clearance of MMA(V)-derived radioactivity from blood was 50 g/h for both doses.

Speciation of urinary arsenic excreted by mice administered MMA(V) or MMA(III) showed differences in their metabolism (Fig. 4). The metabolism of MMA(V) was minimal and that of MMA(III) was extensive. In urine of control mice, DMA(V) ($0.11 \pm 0.02 \mu\text{g/ml}$), DMA(III) ($0.180 \pm 0.003 \mu\text{g/ml}$) and TMAO ($0.052 \pm 0.009 \mu\text{g/ml}$) were detected and may have originated from the mouse diet. After accounting for background levels of urinary arsenicals, less than 10% of the excreted arsenicals for either dose of MMA(V) were in di- and trimethylarsenic forms (Table 2). In contrast, after administration of MMA(III), more than 90% of the excreted arsenicals were in dimethylarsenic forms. The presence of MMA(V) in urine of MMA(III)-treated mice indicates that excreted parent had oxidized at some point in the experiment. An unknown percentage of excreted DMA(III) may have also oxidized to DMA(V) in urine.

Speciation of tissue arsenicals showed that at 2 h post-dosing, administered MMA(V) and MMA(III) were converted to dimethylarsenic (DMA) (Table 3). Because the digestion method used for tissue analysis of arsenicals converts trivalent arsenic to pentavalent arsenic, results are shown as MMA and DMA without regard to oxidation state. TMAO was not detected in the tissues. The highest levels of methylated arsenic (MMA and DMA) at this time point were urinary bladder for both dose groups of MMA(V) and lung of MMA(III)-dosed mice. There was a marked contrast in the tissue distribution of methylated arsenic of MMA(V)- and MMA(III)-dosed mice. In the former, adjusting for background levels of MMA and DMA in tissues, the distribution of methylated arsenic in the form as DMA ranged from 0% in blood to 19% in lung in the low dose group and from 4% in bladder to 25% in lung in the high dose group. In MMA(III)-dosed mice, the distribution of methylated arsenic in the form as DMA ranged from 75% in blood to 100% in bladder, kidney and lung.

The sum of concentrations of arsenic species from the HG-AAS analysis agreed with total arsenic concentrations determined by HG-AFS relatively well as demonstrated in the recovery results. Recovery of arsenic in urine was $104.3 \pm 15.4\%$ and $103.7 \pm 12.1\%$ for 0.4 and 40 mg As/kg MMA(V), respectively. Recovery of arsenic in urine for MMA(III)-treated mice was $104.8 \pm 17.1\%$. Recovery of arsenic in tissues of MMA(III)-treated mice was $103.6 \pm 33.5\%$ and $113.7 \pm 11.7\%$ for kidney and liver, respectively. Recovery of arsenic in kidney was $76.0 \pm 25.7\%$ and $113.0 \pm 20.9\%$ for 0.4 and 40 mg As/kg MMA(V), respectively. Recovery of arsenic in liver was $87.9 \pm 23.6\%$ for 40 mg As/kg MMA(V). Other doses or tissues were not reported because either the tissues were used up in the speciation analysis (bladder) or the level

of arsenic detected in the tissues (blood, liver and lung) was at or below the detection limit of the instrument.

Discussion

Oral administration of MMA(V) to mice results in its rapid absorption, distribution and elimination. The time to peak concentration of MMA(V)-derived radioactivity in blood (1–4 h) of mice was slightly earlier than that observed in hamsters (27 mg As/kg) (6 h), sheep (10 mg As/kg) (5 h) and goats (10 mg As/kg) (5 h) treated similarly (Shariatpanahi and Anderson, 1984; Yamauchi et al., 1988). In humans, MMA(V) (500 µg As) was also rapidly absorbed, based on the percentage of the dose (46%) recovered in urine 4 h after oral administration (Buchet et al., 1981). In the present study, MMA(V)-derived radioactivity was detected in all organs analyzed 0.25 h after administration of MMA(V), which indicates that it distributed rapidly. For both dose levels of MMA(V), 80% of the administered dose was excreted 8 h post-exposure in urine and feces, which also indicates it was rapidly eliminated.

The gastrointestinal absorption of MMA(V) in mice appeared to be dose dependent, with reduced fractional absorption at the higher dose level. By 24 h post-exposure, a significantly higher percentage of the administered dose was excreted in feces by the high dose group. During this same time frame, a significantly higher percentage of the dose was excreted in urine by the low dose group. There was also less than a 100-fold difference in the AUC for the tissue concentration-time profiles between the two doses of MMA(V), although there was a 100-fold difference in the dose administered. At most of the time points where a significant difference in tissue concentration of arsenic was detected, a significantly greater concentration was in the low dose mice.

In hamsters, Yamauchi et al. (1988) observed lower urinary excretion (<35% of the dose) of orally administered MMA(V) (27 mg As/kg) than reported for humans (90% of 500 µg As dose) (Buchet et al., 1981), sheep (53% of 10 mg As/kg dose) and goats (50% of 10 mg As/kg dose) (Shariatpanahi and Anderson, 1984). After ip administration of MMA(V) (27 mg As/kg) in hamsters, 80% of the dose was excreted in urine by 24 h, whereas fecal excretion was 1% (Yamauchi et al., 1988). In contrast, 56% of a po dose of MMA(V) (27 mg/kg) in hamsters was excreted in feces by 24 h (Yamauchi et al., 1988). Thus, it appears that the hamster has relatively lower oral absorption of MMA(V) than other species. The results of parenteral administration of MMA(V) in hamsters also suggest that there is minimal biliary excretion of MMA(V). In rats, it has been recently reported that biliary excretion of iv administered MMA(V) (0.2–0.25 mg/kg) was low (Cui et al., 2004; Suzuki et al., 2004a). Low biliary excretion of administered MMA(V) in the mouse may also be likely and would suggest that absorbed MMA(V) would be preferentially excreted in urine. In mice administered MMA(V) (0.4 or 40 mg As/kg) iv, 4–9% of the dose was excreted in feces and 70–75% in urine by 24 h (Hughes and Kenyon, 1998). Interestingly, administered iAs undergoes biliary transport in the form of an MMA(III) thiol complex in several species, including the hamster (Gregus et al., 2000; Kala et al., 2000) and rat (Cui et al., 2004).

The absorbed dose of MMA(V)-derived radioactivity was rapidly excreted in urine by mice. The rapid urinary excretion accounts for the highest tissue concentrations of MMA(V)-derived radioactivity in kidney and bladder compared to the other organs. Kidney and bladder also had the longest tissue elimination half-lives of MMA(V)-derived radioactivity, which suggests that the absorbed dose is continuously excreted in the urine. Our results agree with those of Yamauchi et al. (1988) who demonstrated that in hamsters dosed po with MMA(V) (27 mg As/kg), kidney had the highest arsenic concentration and the slowest arsenic clearance rate of the organs analyzed. MMA(V) (500 µg As) administered to humans is also rapidly excreted, with approximately 46% and 70% of the dose excreted in urine by 4 and 24 h post-

administration, respectively (Buchet et al., 1981). Clearance (50 g/h) of MMA(V)-derived radioactivity from blood of mice was the same for both administered doses. This suggests that the urinary excretion of absorbed MMA(V) in mice after oral administration was not affected by dose, which is in agreement with data following iv administration of similar doses of MMA(V) in this species (Hughes and Kenyon, 1998).

The level of metabolism of MMA(V) was in sharp contrast to the metabolism of MMA(III) after po administration. For low dose mice, no products attributed to methylation of administered MMA(V) were detected in urine; only a small percentage (1.5%) of the excreted dose was in the form as MMA(III). For high dose mice, approximately 10% of the excreted dose in urine could be attributed to the methylation of administered MMA(V), as DMA(V), DMA(III) and TMAO were detected above background levels. For MMA(III)-treated mice, >90% of the excreted dose in urine was in the form of DMA(V) and DMA(III), which resulted from methylation of administered MMA(III). In tissues of MMA(V)-treated mice, 25% or less of the total methylated arsenic species (MMA + DMA) were due to methylation of administered MMA(V). In tissues of MMA(III)-treated mice, 75–100% of the methylated arsenic species were due to methylation of administered MMA(III). Minimal metabolism of MMA(V), as measured by urinary metabolites, was also reported by our laboratory after iv administration of MMA(V) at the same dose levels and in the same strain of mice (Hughes and Kenyon, 1998). Yamauchi et al. (1988) reported similar observations in the hamster after po administration of MMA(V) (27 mg As/kg). Approximately 5% of the excreted dose was in the form as DMA and <1% in the form as TMAO. Similarly low levels of DMA were also found in tissues of MMA(V)-treated hamsters (Yamauchi et al., 1988). In rats, MMA(V) is excreted in urine primarily intact after iv administration (0.2–0.25 mg/kg) (Cui et al., 2004; Suzuki et al., 2004a). In humans, metabolism of orally administered MMA(V) (500 µg As) to DMA is also low; approximately 13% of the excreted dose of administered MMA(V) was in the form as DMA (Buchet et al., 1981).

Regarding the detection of DMA(III) in urine, it has recently been reported that DMA(III) has been mistaken for a sulfur-containing dimethylarsenic compound (Hansen et al., 2004; Suzuki et al., 2004a, 2004b). These results were based on mass spectral analysis of several products formed from DMA(V) by reacting it directly with sulfur-containing compounds under acidic conditions. This metabolite was detected in urine of sheep fed seaweed, which contains arsenosugars (Hansen et al., 2004). Suzuki et al. (2004a, 2004b) have also detected sulfur-containing dimethylarsenic compounds in liver of rats administered iv DMA(V) (0.5 mg As/kg) or thiol complexes of trivalent monomethyl- or dimethylarsenic (0.5 mg As/kg). The trivalent dimethylarsenic standard we used was dimethylarsine iodide, which forms DMA(III) when dissolved in water. Whether or not the trivalent dimethylarsenic species we detected in urine was DMA(III) or the sulfur-containing dimethylarsenic compound is not known and requires additional studies for verification.

The in vivo MMA(V) and MMA(III) metabolism results of this study and those of others with MMA(V) (Buchet et al., 1981; Cui et al., 2004; Suzuki et al., 2004a, 2004b; Yamauchi et al., 1988) parallel the results from in vitro metabolism studies with these two organic arsenicals (Styblo et al., 1995, 2000). In the in vivo and in vitro studies, metabolism of MMA(V) was minimal, but it was extensive for MMA(III). In studies with rat liver cytosol, MMA(III) was quantitatively metabolized to DMA. More than 95% of MMA(III) added to the cytosolic incubate was methylated to DMA, whereas only 3% of MMA(V) was similarly converted (Styblo et al., 1995). In rat hepatocytes, approximately 90% of MMA(III) (1 µM), but only 5% of MMA(V) (1 µM) was methylated to DMA (Styblo et al., 2000).

There are three potential factors that may limit the in vivo methylation of MMA(V). These may include uptake of MMA(V) into the liver, reduction of MMA(V) and its rapid distribution

to the kidney for elimination. MMA(V) does not appear to be taken up well by the liver, a major site of iAs metabolism (Thomas et al., 2001; Vahter and Concha, 2001). The AUC from the liver concentration-time profiles of MMA(V)-treated mice were only slightly greater (1.1- to 1.4-fold) than the blood AUC, suggesting limited hepatic uptake of MMA(V). Yamauchi et al. (1988) have reported that the concentration of mono- and dimethylarsenic was lower in liver than in blood of hamsters administered MMA(V) (27 mg As/kg) po. If MMA(V) is not taken up into the liver, its methylation is likely to be limited. On the other hand, MMA(III) appears to be more readily taken up than MMA(V) by the liver. In the present study, hepatic levels of DMA were higher in MMA(III)-treated mice (212 ng As/g) than in MMA(V)-treated mice (12 ng As/kg) at a similar administered dose (0.4 mg As/kg) 2 h post-dosing. This suggests that MMA(III), but not MMA(V), was readily taken up by the liver and methylated. Uptake of MMA(III) (1 μ M) by rat hepatocytes was approximately 50% of the total arsenic, while uptake of MMA(V) (1 μ M) was considerably lower (Styblo et al., 2000). Uptake of MMA(III) was also greater than that of MMA(V) by Chinese hamster ovary cells (Dopp et al., 2004). The reason for this difference in hepatic uptake of these two arsenicals is not known. Arsenate (pentavalent iAs), because it is an analogue of phosphate (Dixon, 1997), is transported across membranes by phosphate carriers (Huang and Lee, 1996; Kenney and Kaplan, 1988). However, MMA(V) is not a phosphate analogue (Delnomdedieu et al., 1995), and thus it is unlikely to be transported into cells by phosphate carriers. MMA(V) has a pK_{a1} of 2.6 (Braman et al., 1977), and would be expected to be ionized at physiological pH, thus decreasing its ability to diffuse passively across membranes. Arsenite (trivalent iAs) is transported across membranes by aquaglyceroporins and hexose permeases (Liu et al., 2002, 2004a, 2004b). Whether or not MMA(III) is transported by these mechanisms is not known. What is known is that hepatic uptake of MMA(III) is greater than that of MMA(V), which most likely impacts the extent of their methylation.

According to Challenger (1945), reduction of pentavalent arsenic to a trivalent state is required before methylation can occur. In the present study, less than 12% of the excreted arsenic was in a form that resulted from the initial reduction of administered MMA(V). A mechanism to reduce MMA(V) may not be readily available so that it can be methylated. An MMA(V) reductase has been partially purified from rabbit (Zakharyan and Aposhian, 1999) and human liver (Zakharyan et al., 2001). This enzyme appears to be a member of the glutathione-S-transferase omega (GST-O) family (Zakharyan et al., 2001). Based on immunohistochemical studies, this enzyme has been detected in a number of mammalian species and is widely distributed in a number of tissues (Board et al., 2000; Yin et al., 2001). In vitro studies indicate that this enzyme has a relatively high K_M for MMA(V) (Zakharyan and Aposhian, 1999). The capability of GST-O in reducing MMA(V) in vivo is unknown. Another possibility is that MMA(V) is not an intermediate in the pathway for iAs metabolism as proposed by Hayakawa et al. (in press). MMA(III) is already in the trivalent state, so it can be directly methylated to form DMA(V) as proposed by Challenger (1945). Complexation of MMA(III) with glutathione before methylation would be required by the Hayakawa et al. (in press) pathway. Regardless of the mechanism, quantitative formation of DMA from MMA(III) was observed in vivo in the present study and in vitro by Styblo et al. (1995, 2000).

A major portion of the MMA(V) absorbed into the blood from the gut was rapidly transported to the kidneys and eliminated in urine. This has been observed in mice (Hughes and Kenyon, 1998) and rats (Cui et al., 2004; Suzuki et al., 2004a) after iv administration of MMA(V). If an arsenical is rapidly eliminated from the body, the likelihood of it being methylated is greatly minimized. All of these potential processes, the low hepatic uptake of MMA(V), the low MMA(V) reducing ability within the hepatocyte and the rapid distribution of MMA(V) to kidney and bladder followed by its elimination in urine may explain the low methylation of MMA(V) to di- and trimethylated arsenic species.

In summary, MMA(V) is rapidly absorbed, distributed and eliminated in urine after oral administration in the mouse. The route of excretion of administered MMA(V) was dose dependent, with greater urinary excretion in the low dose group and greater fecal excretion in the high dose group. This and other evidence suggests oral absorption of MMA(V) was dose dependent. Methylation of MMA(V) was minimal and that of MMA(III) was extensive. Arsenic in tissues and urine of MMA(V)-dosed mice was primarily in the form as monomethylarsenic. In contrast, arsenic in tissues of MMA(III)-dosed mice was primarily in the form as dimethylarsenic and that in urine was predominantly di- and trimethylarsenic. Administered dose of MMA(V) did not appear to inhibit its metabolism, as di- and trimethylarsenic were detected in urine of the high dose group, but not in urine of the low dose group. The low methylation and rapid excretion of MMA(V) may partly explain its relatively low toxicity in mammals.

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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

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Fig. 1.

Concentration of MMA(V)-derived radioactivity, based on percentage of dose/g, in tissues of mice over time after po administration of 0.4 (■) or 40 (○) mg As/kg [14C]-MMA(V). The data represent mean ± SD, $N = 4$, except for bladder, 0.4 mg As/kg dose, at 15 min, where $N = 3$. Significant dose effects ($P < 0.05$) on the concentration of MMA(V)-derived radioactivity in tissues were observed; *significantly greater than 40 mg As/kg; † significantly greater than 0.4 mg As/kg.

Fig. 2.

Concentration of arsenic, based on ng As/g, in tissues of mice over time after po administration of 0.4 (■) or 40 (○) mg As/kg [¹⁴C]-MMA(V). The data represent mean ± SD, $N = 4$, except for bladder, 0.4 mg As/kg dose, at 15 min, where $N = 3$.

Fig. 3.

Time course of the excretion of MMA(V)-derived radioactivity in urine and feces of mice after po administration of 0.4 (■) or 40 (○) mg As/kg [^{14}C]-MMA(V). The data represent mean $\pm$ SD, $N = 4$. Significant dose effects ($P < 0.05$) on the cumulative percent dose of MMA(V)-derived radioactivity excreted in urine and feces were observed. *Significantly greater than 40 mg As/kg; †significantly greater than 0.4 mg As/kg.

Fig. 4. Concentration ($\mu\text{g/ml}$) of speciated arsenicals excreted in cumulative 24-h urine of mice administered po 0.4 or 40 mg As/kg MMA(V) or 0.4 mg As/kg MMA(III). Control animals were administered water. Aliquots of urine were directly analyzed by pH selective HG-AAS. The data represent mean $\pm$ SD, $N = 3$ for MMA(V)-treated mice and $N = 4$ for control and MMA(III)-treated mice.

Area under the curve (AUC), terminal half-life ($t_{1/2}$) and ratio of the areas of MMA(V)-derived radioactivity in tissues of mice after oral administration of 0.4 or 40 mg As/kg [^{14}C]-MMA(V)

Tissue	0.4 mg As/kg		40 mg As/kg		Ratio ^a
	AUC ^b	$t_{1/2}$ (h)	AUC	$t_{1/2}$ (h)	
Urinary bladder	0.90 ^c	9.0	56.40	10.9	62.7
Blood	0.16	4.9	11.96	8.3	74.8
Kidney	0.81	15.9	56.16	12.9	69.3
Liver	0.23	4.2	13.37	3.9	58.1
Lung	0.39	4.4	25.11	4.7	64.4

^aRatio represents AUC₄₀/AUC_{0.4}.

^bAUC data and $t_{1/2}$ estimates from Fig. 2.

^cµg-h/g tissue.

Table 2
Distribution of 24 h cumulative urinary arsenicals excreted by mice after po administration of MMA(V) or MMA(III)

Chemical	Dose ^a	% MMA(V)	% MMA(III)	% DMA(V)	% DMA(III)	% TMAO
MMA(V)	0.4	98.2 ± 0.9 ^a	1.8 ± 0.9	—	—	—
MMA(V)	40	89.6 ± 0.6	1.2 ± 0.9	6.2 ± 0.4	1.1 ± 0.7	1.9 ± 1.5
MMA(III)	0.4	6.3 ± 7.8	0.6 ± 0.8	81.5 ± 8.0	10.9 ± 1.5	—

^aData represents net mean % ± SD, after subtraction of arsenical levels in urine of control animals from arsenical levels in dosed animals (data from Fig. 4); N = 3 for MMA(V) samples and N = 4 for MMA(III) samples.

Table 3
Concentration of speciated arsenicals (ng As/g tissue) in tissues of control, MMA(V)- and MMA(III)-exposed mice

Tissue	Chemical	Dose	IAs	MMA	DMA
Urinary bladder	Control	—	21.2	45.4	28.6
	MMA(V)	0.4	41.1 ± 18.6	397.4 ± 288.1	42.8 ± 17.5
	MMA(V)	40	1271.0	25765.9 ± 25353.3	977.2 ± 301.7
Blood	MMA(III)	0.4	ND ^b	29.3	234.3 ± 81.6
	Control	—	1.6	1.0	3.3
	MMA(V)	0.4	1.5 ± 1.8	13.9 ± 6.5	2.5 ± 1.7
Kidney	MMA(V)	40	53.9	866.6 ± 240.9	60.5 ± 27.5
	MMA(III)	0.4	0.6 ± 0.3	12.7 ± 3.9	40.6 ± 1.9
	Control	—	18.1	9.6	15.6
Liver	MMA(V)	0.4	13.7 ± 8.9	68.1 ± 32.7	22.4 ± 14.7
	MMA(V)	40	133.6 ± 53.4	2796.6 ± 1519.4	564.9 ± 259.3
	MMA(III)	0.4	19.3 ± 7.1	6.6 ± 1.4	249.2 ± 50.3
Lung	Control	—	16.9	2.5	13.2
	MMA(V)	0.4	19.5 ± 4.1	23.5 ± 5.7	12.2 ± 5.0
	MMA(V)	40	64.7 ± 16.8	871.5 ± 391.1	215.6 ± 76.2
Lung	MMA(III)	0.4	16.6 ± 13.4	35.4 ± 8.3	211.5 ± 28.3
	Control	—	18.4	11.1	22.7
	MMA(V)	0.4	10.1 ± 6.6	34.3 ± 24.0	28.3 ± 11.1
Lung	MMA(V)	40	26.6 ± 5.5	1156.5 ± 412.7	402.6 ± 40.3
	MMA(III)	0.4	23.4 ± 6.9	8.5 ± 2.1	347.8 ± 69.0

Animals were sacrificed and tissues were removed 2 h after po administration of MMA(V) or MMA(III). Control animals were administered water. Kidney, liver and lung were homogenized in water (100 ng/ml). Aliquots of the tissue homogenate, blood and the whole bladder were digested in 2 M phosphoric acid. Because this procedure converts all tissue arsenicals to the pentavalent oxidation state, the data summarize both pentavalent and trivalent arsenicals. The data represent mean ± SD, *N* = 3–5, except control, where *N* = 2, and numbers without SD, *N* = 1.

^a₁As, inorganic arsenic;

^bND, not detected.