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

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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

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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 (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 multiorgan 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). Carbosorb 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-tube in liquid nitrogen. After 3 min, the liquid nitrogen is removed and the U-tube is heated, thereby separating the arsines on their boiling point. The volatilized arsines are then flushed into a heated multiatomizer and detected with an electrodeless discharge lamp at a wavelength of 197.3 nm.

Aliquots of urine were analyzed without acid digestion. Kidney, liver and lung homogenates were prepared in deionized water at a ratio of 1 g tissue to 5 ml water. Because of their small size, the urinary bladders were digested in phosphoric acid without homogenization. Three milliliters of 2 M phosphoric acid was mixed with no more than 0.025 g of tissue (0.125 ml of homogenate) or 0.01 ml of blood. This mixture was heated at 90 °C in a water bath overnight to digest the sample.

For quantitation of DMA(III) and TMAO in urine, arsines were generated after injection of a urine sample into a flow of deionized water, which was then mixed with 0.75 M Tris–HCL buffer (pH 6) and 1% (w/v) $NaBH_4$ in 0.1% (w/v) NaOH. For quantitation of TMAO in acid-digested tissues, sodium hydroxide (10.9 M, 0.2 ml/ml digest) was first added to cooled tissue digests, to adjust the pH to 6, before trimethylarsine was generated as described above. For quantitation of total DMA (DMA(V)+DMA(III) species), sodium hydroxide (10.9 M, 0.2 ml/ml sample) was added to urine and cooled tissue digests to adjust the pH to 6, followed by pre-treatment with 2% L-cysteine for 70 min to reduce all arsenicals to trivalent forms before dimethylarsine was generated as described above.

Mass spectrometric determination of thioarsenicals

Urine samples were analyzed for dimethylthioarsinic acid (DMTA) and trimethylarsine sulfide (TMAS) using reverse phase chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS). An Agilent 1100 HPLC system (Palo Alto, CA, USA) was used for all chromatography. A Phenomenex (Torrance, CA, USA) Intersil 5 μ ODS-2 (C-18) column with a flow rate of 0.5 ml/min and an injection volume of 100 μl was used. The mobile phase was 2.5 mM tetramethylammonium hydroxide (Alfa, Danvers, MD, USA), 1.1 mM disodium malonate (Sigma) in 5% (wt/wt) methanol (Optima, Fisher Scientific, Pittsburgh, PA, USA), adjusted to pH 6.1 with acetic acid (TraceMetal grade, Fisher Scientific). Urine samples were diluted 10- to 1000-fold for detection with mobile phase in acid-rinsed autosampler vials (Target DP, National Scientific Co., Duluth, GA, USA).

An Agilent 7500a inductively coupled mass spectrometer (ICP-MS) was used for detection (m/z=75) and quantitation of the methylated thioarsenicals. Quantitation of DMTA and TMAS was based on comparison of integrated peak areas from 100 μl sample injections with those of known standards. Three separate standard solutions were used because long-term stabilities (i.e., weeks) of standard mixtures containing DMTA and TMAS have not been verified in a laboratory environment. One standard contained DMA(V), MMA(V) (98% purity, Chem Service, West Chester, PA, USA) and arsenate (Spex Certiprep, Metuchen, NJ, USA). The other two standards contained DMTA or TMAS prepared by bubbling H_2S (generated by reacting FeS and HCl) through a solution containing DMA(V) or TMAO in 18 Ω distilled deionized water (Fricke et al., 2005). The structures of the prepared DMTA and TMAS were determined by reverse phase HPLC-electrospray ionization-mass spectrometry as described by Adair et al. (2007). Identification of the thioarsenicals in mouse urine was by co-chromatography with these known standards of DMTA and TMAS. All standards were prepared on the basis of arsenic content and are analytically traceable to SRM 1640 (trace elements in natural water, NIST, Gaithersburg, MD, USA). Samples were analyzed by standardizing (using the three standard solutions) and verifying standardization after every six samples and at the end of a sample set.

Data analysis

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

Results

DMA(V)-derived radioactivity was detected in urine and all tissues examined by 15 min post-administration, indicating that the processes of absorption, distribution and elimination of this compound occur rapidly in the mouse. The distribution of DMA (V)-derived radioactivity, based on % dose, was widespread throughout the tissues, but the levels were low (Fig. 1). Peak levels of radioactivity were detected 1–2 h post-administration and were highest in liver (high dose—1.27%; low dose—1.81%), followed by kidney, lung and urinary bladder. Significant differences with respect to dose were detected within 2 h post-administration in the distribution of radioactivity to kidney, liver and lung (Fig. 1). In these cases, there was a significantly greater % of the dose detected in the tissues of the low dose group than in tissues of the high dose group. At 24 h, less than 0.05% of the dose remained in the tissues examined, suggesting that this compound is not retained by the mouse.

Tissue concentrations of DMA(V)-derived radioactivity, based on % dose/g, peaked 1–2 h after administration in all tissues at both dosage levels, except for the urinary bladder at the high dosage level (Fig. 2). In the latter case, the peak concentration was at 4 h. The peak concentrations of DMA(V)-derived radioactivity in the kidney, liver, lung and urinary bladder were greater than those in the blood, indicating that the compound had distributed from the blood into these organs. Peak concentrations of radioactivity were greatest in lung and kidney (high dose—2.5% dose/g; low dose—2.2 and 2% dose/g, respectively), followed by liver,

urinary bladder and blood for both dosages of DMA(V). Significant dose effects on the relative concentration of DMA-derived radioactivity were detected in lung, blood, liver and kidney at several time points (Fig. 2). These differences occurred within 4 h post-exposure and in all cases, the relative concentration of DMA(V)-derived radioactivity in tissues of the low dose group was significantly greater than in tissues of the high dose group.

Tissue concentration–time profiles of DMA(V)-derived radioactivity (Fig. 3), based on ng As/ g, appeared parallel for both dosages of DMA(V). The arsenic concentration (ng As/g) in each tissue of the high dose group was consistently greater than that in the low dose group. However, the ratios of high to low dose AUC were less than 100, suggesting that there were dose-dependent effects in the disposition of DMA(V) (Table 1). For both dosage levels, the lung was the tissue with the highest AUC. At the low dose, lung was followed by urinary bladder, kidney, liver and blood. At the high dose, lung was followed by kidney, urinary bladder, liver and blood. The elimination half-life of DMA-derived radioactivity was similar at both dosages of DMA(V) for blood, lung and kidney and ranged from 5 to 8 h (Table 1). The elimination half-life in the urinary bladder had a similar value at the low dose as the latter organs but increased to about 16 h at the high dose. Of all tissues examined, the elimination half-life was longest in the liver: 14 h at the low dose and 38 h at the high dose of DMA(V).

The systemic bioavailability of DMA(V)-derived radioactivity was estimated as 0.88 and 0.48 for the 0.6 and 60 mg As/kg dose, respectively. The clearance of DMA(V)-derived radioactivity from blood was estimated as 33 and 21 g/h for the 0.6 and 60 mg As/kg dose, respectively.

The 24-h cumulative urinary elimination of DMA(V)-derived radioactivity was 65% and 37% of the administered dose for the low and high dose groups, respectively (Fig. 4). The percentage of the dose excreted in urine by the low dose group was significantly greater than that excreted by the high dose group at 8, 12 and 24 h.

The 24-h cumulative fecal excretion of DMA(V)-derived radioactivity was 49% and 71% of the administered dose for the 0.6 and 60 mg As/kg dose groups, respectively (Fig. 4). In contrast to the urinary excretion data, the high dose group excreted a significantly greater percentage of the dose in feces than the low dose group at 8, 12 and 24 h.

Low levels of DMA(V), DMA(III) and TMAO were detected by HG-AAS in the urine of control animals (Table 2). Following administration of DMA(V) or DMA(III) to the mice, DMA(V), DMA(III) and TMAO concentrations in urine increased. An exception to this response was seen in the DMA(V) low dose group, in which excretion of DMA(III) remained at control levels. For DMA(V) at the 0.6 mg As/kg dose, similar concentrations of DMA(V) and TMAO were found in urine. At the higher dose of DMA(V), levels of parent compound and of the two metabolites exceeded those found in the low dose group. MMA(V) was also detected in this group and is most likely from that in the dosing solution. In the DMA(III)-treated group, TMAO was the predominant urinary metabolite and similar levels of DMA(III) and DMA(V) were found. The presence of DMA(V) in the urine of the DMA(III)-treated group shows that oxidation of DMA(III) occurred spontaneously *in vivo*, as a component of metabolism or in voided urine.

The methylated thioarsenicals, DMTA and TMAS, were excreted in urine of control and DMA (V) and DMA(III)-treated mice (Table 3). In DMA(III)- and DMA(V)-exposed mice, concentrations of DMTA and TMAS in urine were 6- to 50-fold greater than those found in control urine. Urinary concentrations of DMTA and TMAS were similar in DMA(V)- and DMA(III)-treated mice, suggesting that the dosage level or oxidation state of arsenic did not affect the excretion of DMTA or TMAS.

DMA was the predominant arsenical in tissues of mice 2 h after oral administration of DMA (V) or DMA(III) (Table 4). Because tissues were solubilized in phosphoric acid as a prelude to HG-AAS analysis, the oxidation state of arsenic present in various arsenicals in tissues cannot be determined. Therefore, the values for iAs and mono- and dimethylated arsenic are reported as total speciated arsenic (iAs(III+V), MMA(III+V) and DMA(III+V)). TMAO was detected consistently in kidney for both doses of DMA(V) and in liver, lung and urinary bladder of the high dose group. TMAO was detected in the liver and lung of one sample from the DMA (III)-treated group and low dose DMA(V) group in the liver. At 2 h, the lung was the organ with the highest concentration of arsenic and the predominant species was DMA. The MMA that was detected is most likely from that within the original DMA(V) dosing solution, which contained approximately 9% MMA(V).

Discussion

The absorption, distribution and excretion of DMA(V) by mice were rapid after oral administration. DMA(V)-derived radioactivity was detected in urine and all tissues by 15 min post-treatment. A similar pattern of rapid absorption, distribution and excretion of radioactivity was observed in mice of the same strain and sex after oral treatment with [^{14}C]-MMA(V) (Hughes et al., 2005), a metabolic precursor of DMA(V).

The oral absorption of DMA(V) appears to be dose dependent in mice with reduced fractional absorption at the higher dosage. This also occurs in mice with orally administered MMA(V) (Hughes et al., 2005). In the present study, with increased administered dose of DMA(V), the systemic bioavailability decreased, the ratio of the tissue AUCs based on dose were less than 100-fold and there was increased fecal excretion and decreased urinary excretion of DMA(V)-derived radioactivity. Also, at the times where there were significant dose-related differences in tissue concentration of radioactivity, the concentrations in the low dosage group exceeded those of the high dosage group.

Excretion of orally administered DMA(V) occurs rapidly in the mouse. In the present study, approximately 75% of either dose of DMA(V) was excreted in urine and feces within 8 h of treatment. With MMA(V), 80% of an oral dose (0.4 or 40 mg As/kg) was excreted in urine and feces within 8 h (Hughes et al., 2005). Our DMA(V) data are also consistent with other published results in mice, which reported that 95% of an oral dose of DMA (V) (0.4 or 40 mg As/kg) was excreted in urine and feces by 24–48 h (Marafante et al., 1987; Vahter et al., 1984). However, a major difference was the greater percentage of urinary TMAO that was detected in the present study (18–49%) than reported by Marafante et al. (1987) (5%). This difference may be due to strain and sex of the mouse model used and analytical sensitivity between methods used by Marafante et al. (1987) and those used in the present study. Humans also absorb and excrete DMA(V) rapidly. In volunteers, approximately 57–65% of an oral dose of DMA(V) (~7 to 100 μg As/kg) was excreted in urine within 24 h (Buchet et al., 1981; Marafante et al., 1987).

The current study found that there was a dose-dependent decrease in the percentage of DMA (V) excreted in urine and a concomitant dose-dependent increase in the percentage of DMA (V) excreted in feces. This pattern is consistent with findings in MMA(V)-treated mice (Hughes et al., 2005). In rats, biliary excretion of DMA(V) or MMA(V) is less than 1% of the dose after intravenous administration (Cui et al., 2004; Gregus et al., 2000; Suzuki et al., 2004a). Similar data on the biliary excretion of DMA(V) and its metabolites in the mouse are lacking. Fecal excretion of DMA(V) administered intravenously to mice at the same dosages as in the present study is less than 5% of the dose (Hughes and Kenyon, 1998), suggesting that biliary excretion of administered DMA(V) is limited in this species. Urinary excretion of DMA(V) administered intravenously to mice is not affected by dose (Hughes and Kenyon, 1998). The latter

observations and present data suggest that the radioactivity detected in feces of mice treated orally with [^{14}C]-DMA(V) is likely unabsorbed parent compound and also suggests that gastrointestinal absorption of DMA(V) was dose dependent.

Accumulation of DMA in lungs of DMA(V)-treated mice suggests a particular affinity of this organ for dimethylated arsenicals. For both doses of DMA(V), the lung had the highest peak concentration of DMA(V)-derived radioactivity and the highest AUC among the tissues. In addition, the lung had the highest concentration of total DMA among all HG-AAS analyzed tissues from DMA(V)- and DMA(III)-treated mice. The accumulation of DMA in lung also occurs in mice after intravenous administration of this compound (Hughes et al., 2000; Vahter et al., 1984). Following acute oral exposure, DMA(V)-derived radioactivity in the lung disappeared quickly from this tissue, with a terminal half-life of 6 h. However, the short residence of DMA in the lung is sufficient to produce an adverse effect. An acute oral dose of DMA(V) (1.5 g/kg, sodium salt) induces oxidative damage in mouse lung by 6 h post-administration (Yamanaka et al., 1991). Also, DMA(V) in drinking water (400 ppm) promotes lung tumor development in the A/J mouse strain (which is spontaneously susceptible to pulmonary tumors) (Hayashi et al., 1998) and the ddY mouse strain pre-treated with 4-nitroquinoline 1-oxide (Yamanaka et al., 1996).

Rats fed a diet containing 100 ppm of DMA(V) develop urothelial cell carcinomas after a 2-year exposure (Arnold et al., 2006). In contrast, mice fed up to 500 ppm DMA(V) in the diet do not develop treatment-related tumors at any site (Arnold et al., 2006). DMA(III), a reduced metabolite of DMA(V), has been postulated to be the toxic moiety that elicits the carcinogenic effect in DMA(V)-exposed rats (reviewed in Cohen et al., 2006). Cohen et al. (2002) reported that the LC_{50} of DMA(III) in rat and human urothelial cell lines to be less than 1 μM; the LC_{50} for either DMA(V) or TMAO in these cell lines was in the millimolar range. Similarly, in Urotsa cells, a human SV-40 transformed urothelial cell line, the LC_{50} for DMA(III)-glutathione complex was 14 μM and DMA(V) was inactive at the concentrations tested (Styblo et al., 2000). Thus, dimethylated arsenic in the trivalent oxidation state is highly cytotoxic, exceeding both DMA(V) and TMAO in potency. In the present study, the concentration of DMA(III) in urine of the high dose DMA(V)-treated mice was approximately 90 μM. This far exceeded the concentration of DMA(III) (1.4 μM) found in urine of rats fed a diet of 100 ppm of DMA(V) for 24 h (Lu et al., 2003). The lower concentration of DMA(III) in urine of DMA(V)-treated rats might reflect oxidation of trivalent arsenic occurring during sample collection, shipment and processing. It may also be based on administered dose because the rats exposed to 100 ppm DMA(V) received approximately 5.4 mg As/kg (assuming 80 g rat and 10 g food consumed/day/100 g body weight), whereas the mice in the present study received approximately a 10-fold greater dose. Data in the literature to allow a direct comparison between levels of metabolites in mouse and rat urine after exposure to DMA(V) are somewhat sparse, particularly in regard to the more unstable or difficult to detect metabolites. Such a comparison would be of interest because of the differences in responsiveness between rat and mouse urothelium observed during lifetime exposure to DMA (V) in the diet (Arnold et al., 2006). In addition, the proposed mode of action for DMA(V)-induced bladder cancer in rats is cytotoxicity and regenerative proliferation which requires prolonged and relatively continuous exposure to the putative toxic agent. Thus, any such comparison to be meaningful needs to be made in animals whose urinary metabolite excretion has reached steady state levels, which would take place in a few days in the mouse but would require weeks in the rat. Data currently available allow one to propose but not ascribe with certainty causation of the lesions in rat urothelium to DMA(III) or one of the other recently identified methylated thioarsenic metabolites.

The concentration of urinary TMAO was 32 μM and 740 μM in the low and high dose groups of DMA(V), respectively. These data show that the mouse is quite capable of reducing and

methylating DMA(V) to TMAO. However, a fair percentage of the excreted arsenic was in the form of parent DMA(V) (> 50%). Adair et al. (2007) have reported that rats exposed to 100 ppm of DMA(V) in drinking water for 14 days excrete a TMAO concentration of 1200 μM. This value is consistent with that reported for rats treated with an acute oral dose of 50 mg/kg DMA (V) (Yoshida et al., 1997). The urinary concentration of TMAO excreted at 8 h by the acutely treated rats was 2000 μM. The high concentration of TMAO in mouse and rat urine after DMA(V) treatment is consistent with the formation of relatively high concentrations of DMA(III) (90 μM in the high dose DMA(V) group) as an obligate intermediate in the pathway for oxidative methylation of DMA(V) (reviewed in Thomas et al., 2007).

The metabolism of DMA(V) appears to be affected by administered dose. At the low dose of DMA(V) (0.6 mg As/kg), approximately 50% of the excreted arsenic was TMAO. With a 100-fold increase in administered dose, 20% of the urinary excreted arsenic was in the form of metabolites (DMA(III) and TMAO). This would suggest that the potential uptake of DMA (V) by arsenic metabolizing cells was saturated, or that the reduction and methylation of this arsenical in these cells was similarly affected. Marafante et al. (1987) reported that in mice 80–85% of an acute oral dose of DMA(V) (40 mg As/kg) was eliminated as unmetabolized DMA (V). Some of this excreted dimethylated arsenic may have been DMA(III), as the analytical techniques to speciate trivalent and pentavalent DMA were not developed at the time this paper was published. The important observation is that the percentage of methylated DMA(V) decreases as DMA(V) dose increases.

The detection of unknown arsenic metabolites in urine and feces of DMA(V)-exposed rats and mice has been reported in several studies (Hughes and Kenyon, 1998; Marafante et al., 1987; Suzuki et al., 2004a; Yoshida et al., 1997, 1998, 2001, 2003). These metabolites were in addition to DMA(III) and TMAO and some contained one or two sulfur atoms (Yoshida et al., 2003). Hansen et al. (2004) reported that the methylated thioarsenical, DMTA, had long been misidentified as DMA(III). In addition, TMAO, a product of DMA(V) metabolism, is a precursor of one of the metabolites (Kuroda et al., 2004). Intestinal bacteria are thought to have a role in the formation of some of these metabolites (Yoshida et al., 2001; Kuroda et al., 2004). However, two of the metabolites have been detected in livers of rats administered DMA (V), DMA(III), MMA(V) or MMA(III) intravenously and in incubations of DMA(III) with rat liver supernatants (Suzuki et al., 2004b). The two metabolites are dimethylthioarsinous acid and DMTA. More recently, mono-and dimethylated thioarsenicals have been detected in urine of rats and hamsters administered arsenite (Naranmandura et al., 2007). Raml et al. (2007) have also recently reported that DMTA is a metabolite found in urine of Bangladeshi women who live in an area with high levels of iAs in the drinking water. Methylated thioarsenic compounds, some of which were detected in the urine of mice in the present study, are of interest, not only because of their chemical structure, but also because of their potential for toxicity. DMTA and the unknown sulfur arsenic compound formed by bacteria from DMA(V) in the presence of cysteine are more cytotoxic than DMA(V) (Kuroda et al., 2004; Raml et al., 2007). The identification of these methylated thioarsenicals *in vivo* and in excreta demonstrates the complexity of arsenic metabolism and its potential role in arsenic toxicity.

A limitation of this study is the number of animals used, particularly in the metabolism studies. In the dosimetry study, the coefficient of variation (CV) in the distribution and concentration data averaged 26% (mean range 16–51%), indicating that the overall variability of these data was reasonable. None of the standard deviations exceeded the mean value (CV > 100%). Much greater variability was observed in the metabolism study, particularly in the analysis of iAs in the tissues. Several of these CV values for iAs in control and treated animals exceeded 100%. However, in the analysis of parent (DMA(V) or DMA (III)) or metabolite (DMA(III), TMAO, DMTA or TMAS), the majority of the CV values, although high, were less than 100%.

Certainly the addition of more animals to this portion of the study would have reduced the variability in the data.

Because the dimethylated arsine generated from DMTA coelutes with the arsine generated from DMA(III), it is possible that urinary DMTA contributes to the concentration of DMA(III) as determined by HG-AAS. The contribution of DMTA to the estimated concentration of DMA(III) is limited by its low efficiency of arsine generation in the presence of L-cysteine (~35%) compared to DMA(III) (Hernandez-Zavala et al., in press). The concentration of DMTA in urine was estimated as 3 μM using HPLC-ICP-MS and suggests that the error in estimation of DMA(III) in urine by HG-AAS would be fairly small.

In summary, dose affected the absorption, metabolism, distribution and excretion of DMA(V) administered orally to mice. These processes of DMA(V) disposition occurred rapidly. DMA (V) was metabolized to DMA(III), TMAO, DMTA and TMAS, all of which were detected in urine. DMA(III) was metabolized to TMAO, DMTA and TMAS, which were also detected in urine. Parent compound (DMA(V) and DMA(III)) was also detected in urine, indicating these arsenicals are not completely metabolized but are also mobilized for excretion. Administered dose also affected the metabolism of DMA(V), as a lower percentage of the dose was metabolized in the high dose group. The lower absorption and metabolism of high doses of DMA(V) as well as its rapid excretion may partly explain the low acute toxicity of this organic arsenical.

Acknowledgements

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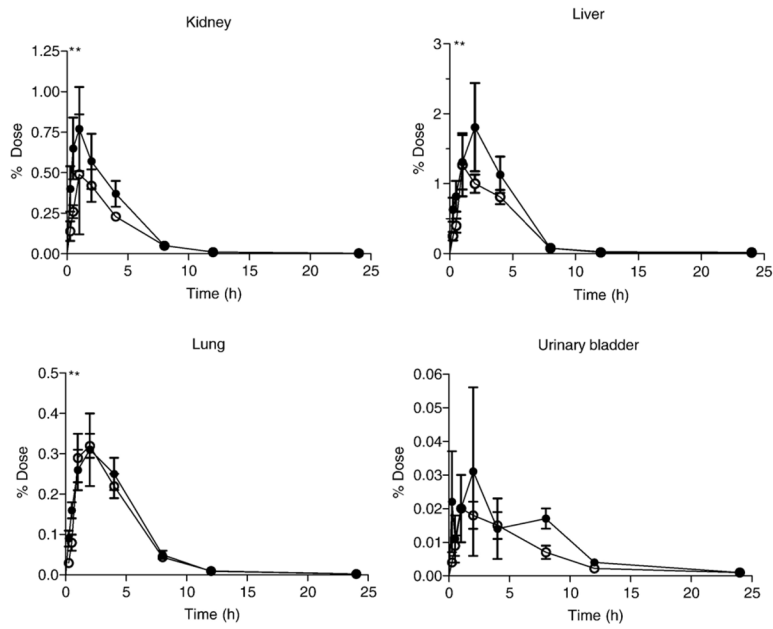
Fig. 1.
Distribution of DMA(V)-derived radioactivity (% dose) in tissues of mice after po administration of 0.6 (●) or 60 (○) mg As/kg [^{14}C]-DMA(V). The data represent mean±SD, N=4. Significant dose effects ($p<0.05$) on the concentration of DMA(V)-derived radioactivity in tissues were observed; *, significantly greater than 60 mg As/kg.
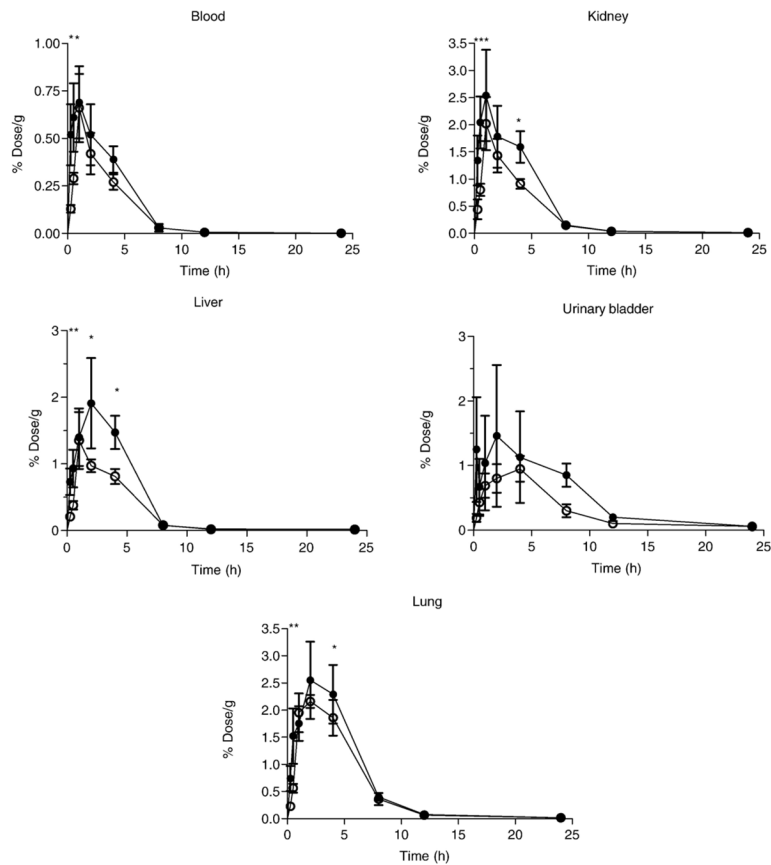
Fig. 2.
Concentration–time profiles of DMA(V)-derived radioactivity, based on % dose/g, in tissues of mice after po administration of 0.6 (●) or 60 (○) mg As/kg [^{14}C]-DMA(V). The data represent mean±SD, N=4. Significant dose effects ($p<0.05$) on the concentration of DMA(V)-derived radioactivity in tissues were observed; *, significantly greater than 60 mg As/kg.
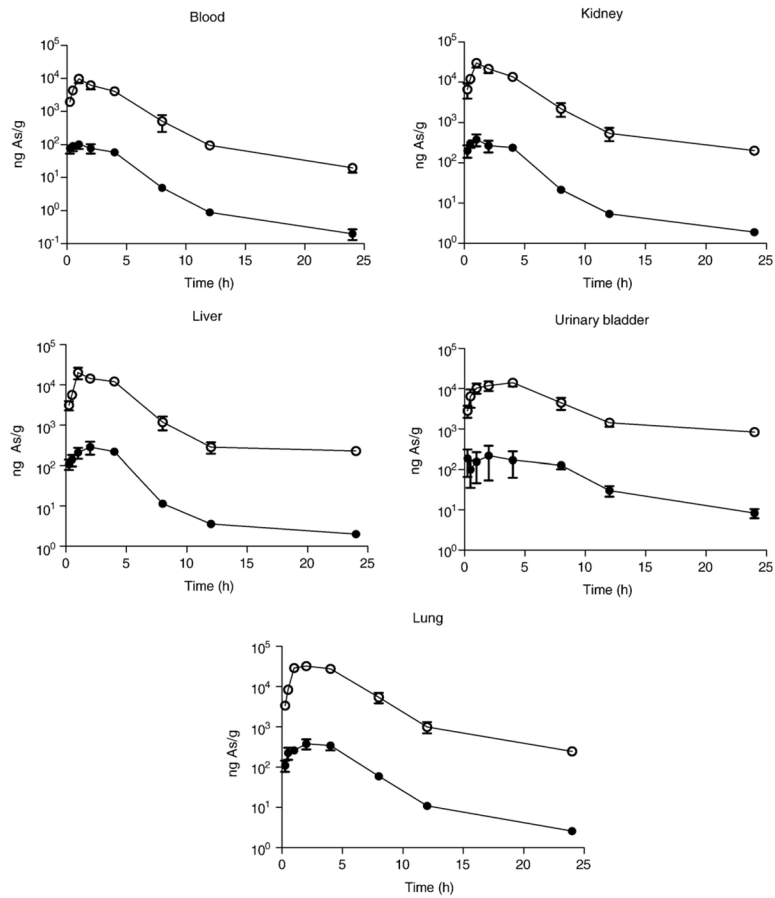
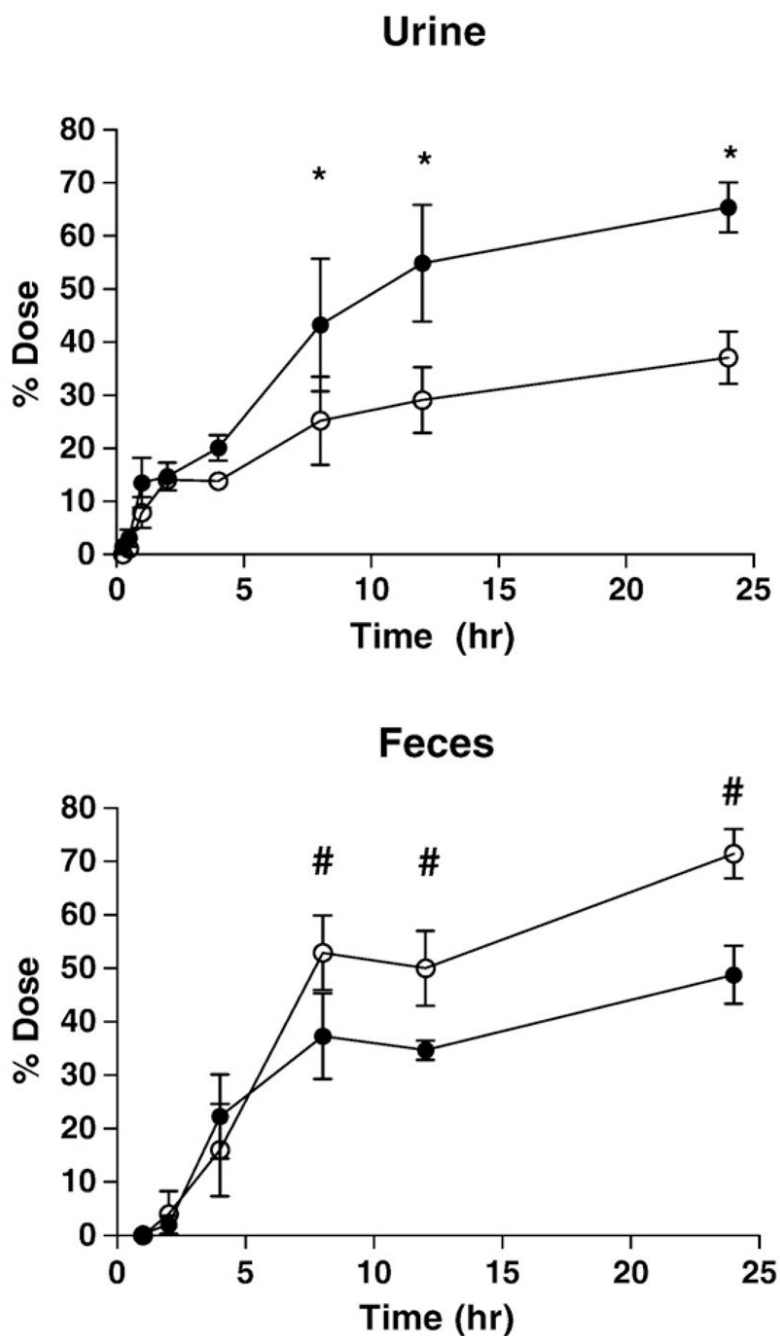**Fig. 3.**
Concentration–time profiles of DMA(V)-derived radioactivity, based on ng As/g, in tissues of mice after po administration of 0.6 (●) or 60 (○) mg As/kg [^{14}C]-DMA(V). The data represents mean±SD, N=4.

Urine

Feces

Fig. 4.
Cumulative excretion of DMA(V)-derived radioactivity in urine and feces of mice after po administration of 0.6 (●) or 60 (○) mg As/kg [^{14}C]-DMA (V). The data represents mean±SD, N=4. Significant dose effects (p<0.05) on the cumulative % dose of DMA(V)-derived radioactivity excreted in urine and feces were observed. *, significantly greater than 60 mg As/kg; #, significantly greater than 0.6 mg As/kg.

Table 1

Area under the curve (AUC), terminal half-life ($t_{1/2}$) and ratio of the AUCs of DMA(V)-derived radioactivity in tissues of mice after oral administration of 0.6 or 60 mg As/kg [^{14}C]-DMA(V)

Tissue	0.6 mg As/kg		60 mg As/kg			
	AUC [a]	$t_{1/2}$ (h)	AUC	$t_{1/2}$ (h)	Ratio [b]	
Urinary bladder	1.8 [c]	6.5	105.9	15.5	58.8
Blood	0.4	5.5	34.1	5.3	85.2
Kidney	1.7	8.0	116.6	8.4	68.6
Liver	1.4	14.1	84.8	37.8	60.6
Lung	2.3	5.8	189.3	6.0	82.3

[a] AUC data and $t_{1/2}$ estimations from Fig. 3.

[b] Ratio represents AUC$_{60}$/AUC$_{0.6}$.

[c] μg h/g tissue.

Table 2

Speciated di- and trimethyl arsenicals (µg As/ml) detected by HG-AAS in 24-h cumulative urine following oral administration of DMA(V) or DMA(III)

Chemical	Dose (mg As/kg)	DMA(V)	DMA(III)	TMAO
Control	—	0.02±0.03 [a]	0.03±0.02	0.16±0.17
DMA(III)	0.6	1.52±2.56	1.48±0.57	2.61±1.17
DMA(V)	0.6	2.99±1.86	0.14±0.08	2.38±1.53
DMA(V) [b]	60	242.93±74.34	6.83±4.65	55.36±31.01

Control levels of iAs were 0.003±0.003 µg As/ml.

[a] Mean±SD, *N*=3.

[b] MMA(V) levels were 5.58±9.68 µg As/ml.

Table 3

Concentration of methylated thioarsenicals (μg As/ml) detected by HPLC-ICP-MS in 24-h cumulative urine of control and DMA(V) or DMA(III)-treated mice

Chemical	Dose (mg As/kg)	DMTA	TMAS
Control	—	0.010±0.002 [a]	0.003±0.001
DMA(III)	0.6	0.10±0.05	0.02±0.01
DMA(V)	0.6	0.16±0.10	0.15±0.16
DMA(V)	60	0.23±0.19	0.14±0.09

[a]Mean±SD, *N*=3.

Table 4

Concentration of speciated arsenicals (ng As/g tissue) in tissues of control and DMA(V)- or DMA(III)-treated mice 2 h after oral exposure

Tissue	Chemical	Dose	iAs [a,b]	MMA [b]	DMA [b]	TMAO
Blood	Control	–	0	5.50±5.26 [c]	19.00±32.23	ND [d]
	DMA(III)	0.6	0.23±0.39	11.87±1.73	65.28±9.8	ND
	DMA(V)	0.6	3.22±5.58	5.02±1.86	36.45±11.45	ND
	DMA(V)	60	0.31±0.54	459.24±119.27	1641.39±377.4	ND
Kidney	Control	–	2.82±2.94	13.0±3.09	5.17±2.24	ND
	DMA(III)	0.6	0.57±0.99	19.51±3.76	246.27±21.51	ND
	DMA(V)	0.6	0	0	68.75±29.12	0.23±0.15
	DMA(V)	60	0	514.38±146.97	10077.95±2779.86	0.42±0.29
Liver	Control	–	0.30±0.52	6.81±1.4	8.01±2.23	ND
	DMA(III)	0.6	17.06±29.54	11.91±1.13	220.59±22.28	7.93 [e]
	DMA(V)	0.6	7.07±7.53	5.38±4.86	111.05±30.93	7.46 [e]
	DMA(V)	60	1.41±2.44	226.0±68.89	7665.83±1606.97	821.83±385.03
Lung	Control	–	4.91±7.13	0	13.64±6.81	ND
	DMA(III)	0.6	3.63±5.41	24.72±1.18	683.46±16.94	8 [e]
	DMA(V)	0.6	0.15±0.26	10.21±0.01	199.64±61.02	ND
	DMA(V)	60	0	1231.63±41.5	21413±7966.05	291.3±63.42
Urinary bladder	Control	–	58.82±32.16	97.91±44.26	17.95±9.4	ND
	DMA(III)	0.6	30.08±31.39	173.05±287.25	305.13±66.34	ND
	DMA(V)	0.6	18.32±9.31	10.8±10.41	71.17±34.09	ND
	DMA(V)	60	0	365.44±322.21	7906.95±1796.36	294.01±70.44

[a] Inorganic arsenic.

[b] Trivalent+pentavalent arsenical.

[c] Mean±SD, N=3.

[d] Not detected.

[e] N=1, peaks from other chromatograms could not be reliably quantitated.