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Metabolism and disposition of arsenic species after repeated oral dosing with sodium arsenite in drinking water. II. Measurements in pregnant and fetal CD-1 mice

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ABSTRACT

Arsenic is ubiquitous in the earth's crust, and human diseases are linked with exposures that are similar to dietary intake estimates. Metabolic methylation of inorganic arsenic facilitates excretion of pentavalent metabolites and decreases acute toxicity; however, tissue binding of trivalent arsenic intermediates is evidence for concomitant metabolic activation. Pregnant and fetal CD-1 mice comprise a key animal model for arsenic carcinogenesis since adult-only exposures have minimal effects. This study evaluated inorganic arsenic and its metabolites in pentavalent and trivalent states in blood and tissues from maternal and fetal CD-1 mice after repeated administration of arsenite through drinking water. After 8 days of exposure, DMA species were ubiquitous in dams and fetuses. Despite the presence of MMA^{III} in dams, none was observed in any fetal sample. This difference may be important in assessing fetal susceptibility to arsenic toxicity because MMA production has been linked with human disease. Binding of DMA^{III} in fetal tissues provided evidence for metabolic activation, although the role for such binding in arsenic toxicity is unclear. This study provides links between administered dose, metabolism, and internal exposures from a key animal model of arsenic toxicity to better understand risks from human exposure to environmental arsenic.

1. Introduction

A large and expanding body of scientific evidence supports an important role for the toxic consequences of environmental exposure to arsenic species in several important human disease states, including cardiovascular, metabolic (e.g., diabetes mellitus), and neurological disorders, and cancer. Much of the evidence linking arsenic exposures to human diseases comes from epidemiological associations in the developing world where high daily intake from elevated levels in drinking water, often reaching into the mg/L (ppm) range. A major challenge for risk assessment is in extrapolating from associations observed in areas with high arsenic exposure, with estimated daily intakes up to 50 μg/kg bw, to populations in the developed world where exposure to arsenic is mainly through the diet, and daily exposures are typically approximately 100-fold lower (European Food Safety Authority, 2009; Centers for Disease Control and Prevention, 2017).

Of special interest in the etiology of arsenic-related diseases is the role for perinatal exposures, because early life stages can be more susceptible to toxicants from the unique targets for disruption expressed during fetal/infant development and/or metabolic and physiologic insufficiencies that can lead to higher internal exposures to toxic species. Evidence linking early life exposures to environmental arsenic species with diseases later in life includes epidemiological associations and results in experimental animal models (reviewed in Farzan et al., 2013 and Bommarito and Fry, 2018). The most robust linkages have been shown for carcinogenesis, in part from a unique exposure scenario from a community in northern Chile where a well-defined change in the sole drinking water source from 1958 to 1970 provided a precise exposure window from which to evaluate the role of early exposures to high levels of arsenic (up to 860 ppb or ~40 μg/kg bw/d) on cancer incidences. Now, > 40 years after the in utero and early-life arsenic exposures, lung, bladder, and kidney cancer incidences are markedly higher than a matched control population from an adjacent region in Chile with consistently low arsenic exposures (Steinmaus et al., 2014; Smith et al., 2018).

Additional support for a perinatal basis of arsenic-induced carcinogenesis comes from experimental animals. Although arsenic is generally without carcinogenic effects when tested in standard adult animal models (International Agency for Research on Cancer, 2004; International Agency for Research on Cancer, 2012), exposure of CD-1

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mice to high levels of sodium arsenite from drinking water either in utero-only (42.5 and 85 ppm or approximately 9.6–19 mg/kg bw/d; Waalkes et al., 2003) or "whole-life" (6–24 ppm or approximately 1.4–5.4 mg/kg bw/d starting in utero until 2 years; Tokar et al., 2011a) produced increased incidences of cancer in several organs (e.g., liver, lung, ovary). Evidence for inorganic arsenic-induced transformation of human stem cells to a cancerous phenotype provides mechanistic support for enhanced carcinogenicity from early life exposure (reviewed in Tokar et al., 2011b).

Metabolism following the ingestion of inorganic arsenic appears inextricably linked with its toxicology. Methylation by arsenite methyltransferase (As3MT; Dheeman et al., 2014) appears to facilitate urinary excretion and to be protective against arsenite toxicity (Currier et al., 2016). However, the concomitant formation of highly reactive trivalent arsenic species, methylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}), and their binding to cellular thiols appear to be central to the toxic effects. A recent publication described the production and binding of trivalent arsenic species throughout the body after controlled dosing with low doses of arsenite in adult CD-1 mice (Twaddle et al., 2018). That study showed that dose was a key determinant in the metabolism of arsenite and the magnitude of trivalent arsenic species binding. The non-linearity between dose and formation/binding of reactive trivalent species suggested that animal models using doses far above human exposures were likely to overestimate the putative toxic effects. That study also showed that reduction of pentavalent arsenic species by glutathione and complexation of trivalent arsenic species with cellular thiols were particularly important in relating metabolism in the liver with exposures to putative toxic arsenic intermediates in extrahepatic tissues.

The current study continues the earlier line of investigation by using repeated dosing of pregnant CD-1 mice with low doses of sodium arsenite to evaluate metabolism and disposition of arsenic species in dams and fetuses throughout pregnancy and to quantify maternal and fetal exposure to reactive intermediates that are capable of toxic interactions. The dosing strategy used drinking water containing sodium arsenite (1 ppm as arsenic) with predicted total daily intake in the range of 100–200 μg/kg bw. This concentration was chosen such that individual drinking events throughout the day would produce acute intakes similar to a 50 μg/kg bw dose, above which non-linear pharmacokinetics were observed previously in gavage-treated adult female CD-1 mice (Twaddle et al., 2018). This concentration is also similar to those used recently for arsenite carcinogenesis in mice (Waalkes et al., 2014) and the upper bounds of drinking water contamination by arsenic affecting highly exposed human communities across the world (US Environmental Protection Agency, 2010). A major goal of this study was to describe the fetal exposures to all potentially toxic arsenic species to understand if any particular susceptibility is related to immaturity of metabolic and excretory capabilities in perinatal animal models under dosing conditions that are closer to typical human exposures than previously reported (43–85 ppm, Devesa et al., 2006; 10–30 ppm, Jin et al., 2006).

2. Methods

2.1. Reagents and standards

Hydrogen peroxide (30%) was purchased from Fisher Optima (Thermo Fisher, Waltham, MA); ammonium phosphate dibasic from Sigma-Aldrich (St. Louis, MO); MilliQ-H_2O (18 MΩ) from Millipore (Billerica, MA); and 30 kDa molecular weight cutoff centrifuge filters (30 kD MWCO) from EMD Millipore (Darmstadt, Germany); . Blood was collected in EDTA-coated plasma separator tubes (MiniCollect, Greiner Bio-One, Monroe, NC) ranging in size from 0.25 to 1 mL.

NIST-certified solutions (standard reference materials, SRMs) of arsenite and arsenate were purchased from SPEX (Metuchen, NJ). MMA^V (disodium methyl arsenate hexahydrate) and DMA^V (dimethylarsinic acid) were purchased from Chem Service (West Chester, PA), sodium arsenite was purchased from Lab Chem (Zelienople, PA), and all solutions were prepared by accurately weighing a portion and diluting with MilliQ-H_2O. All dilutions were prepared in dark, polypropylene bottles and stored at 4 °C. All standards were prepared on the basis of elemental As concentration (75 g/mol) and analyzed by infusion into the ICP/MS to ensure an equal As concentration, using arsenate as the reference (NIST SRM 1640A, trace elements in natural water; Gaithersburg, MD).

2.2. Liquid chromatography

Ion exchange LC was performed using a Thermo UltiMate 3000 HPLC system (Thermo Scientific, Germering, Germany) consisting of a pump and autosampler. A Hamilton PRP-X100 column (4.1 × 250 mm, 10 μ particle size, Hamilton, Reno, NV), with an isocratic mobile phase consisting of 98% 10 mM ammonium phosphate (pH 8.25, prepared daily) and 2% methanol (Thermo Fisher Scientific) was used for analyte separation/speciation. The column effluent was directed through a 10-port switching valve (Rheodyne/IDEX, Lake Forest, IL) that was used to introduce a post-column standard addition of arsenate to provide signal normalization throughout every sample set, as described previously (Twaddle et al., 2018).

2.3. Mass spectrometry

A Thermo X-Series II ICP-MS (Thermo Electron, Bremen, Germany), equipped with a microflow nebulizer and Peltier-cooled spray chamber maintained at 2 °C (PC3, Elemental Scientific, Omaha, NE), was used to monitor elemental arsenic (m/z 75).

2.4. Sample calibration curve

Quantification of each sample set used a series of arsenic standards (arsenite, arsenate, MMA^V, DMA^V) in 10 mM ammonium phosphate (pH 8.25) at defined concentrations to prepare a daily calibration curve. Typically, these standards consisted of a blank along with 3–5 concentrations over a range of 0.05–20 ng/mL As. Linear responses were consistently observed ($R^2 > 0.999$). A typical sample set consisted of calibration standards, a buffer blank, matrix blanks, matrix spikes at multiple concentrations, and incurred samples. Standards were interspersed throughout the sample set to monitor ICP/MS and chromatographic performance.

2.5. Method validation

Method validation consisted of spiking plasma or erythrocytes with three concentrations of mixed arsenic standards and preparing each concentration in quadruplicate (0.1, 1.0, and 10.0 ng/mL) as described previously (Twaddle et al., 2018). Similarly, tissues were spiked at 5, 10, and 100 ng/g with mixed arsenic standards and homogenized with 10 mg-equivalent aliquots analyzed in quadruplicate on separate days as described previously (Twaddle et al., 2018).

In addition to accuracy and precision measurement from spiked samples, an additional evaluation of previously analyzed erythrocyte and plasma samples from dosed mice (n = 2) was performed. In plasma, the within sample variability for DMA^V was 3.1–3.2% and the repeatability from the previous measurement was 103–109%. For erythrocytes, within sample variability was 4.3–12% and repeatability was 105–159% for DMA^V. For DMA^{III}, the respective values were 4.0–4.4% and 109–117%, and for MMA^{III}, the respective values were 5.2–8.9% and 106–127%. The higher values observed for within sample variability and repeatability likely reflect sample handling differences (e.g., pipetting and frothing with H_2O_2) encountered with highly viscous erythrocytes (Twaddle et al., 2018).

Method detection limits for arsenic species, which reflect both the