Arsenic metabolism, excretion, and toxicity

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Arsenic is a global, naturally present pollutant found in drinking water that is consumed by millions of people throughout the world. Humans have evolved an arsenic methyltransferase present in high levels in the liver to expedite its excretion from the body. Most organisms have this enzyme. The enzyme generates monomethyl (MMA) and dimethyl arsenic (DMA). These methylated species are considered more toxic than arsenite, but they bind less tightly to proteins and thereby are excreted more rapidly from the body. Individuals who have been chronically exposed to arsenite were found to possess advantageous genotypes to metabolize arsenites. However, higher expression of AS3MT was found to yield more toxic MMA but lower amounts were still capable of metabolizing arsenite to DMA. This likely reflects the complex metabolic processes involving not only AS3MT, but antioxidants and upstream regulators of AS3MT, potentially transcription factor NRF2. This article discusses arsenic metabolism, excretion, toxicity, and the evolution of this enzyme across various species. The fact that so many organisms have evolved to possess this arsenite metabolizing enzyme is indicative of the widespread and ancient pollution of our drinking water by arsenite.

Keywords: Arsenite, metabolism, arsenite methyltransferase,

1.Introduction

Arsenic is a metalloid found naturally in rocks and soil and is one of the major world-wide contaminants of drinking water. Arsenic has also been used as a wood preservative, pesticide, and also as a chemotherapeutic agent to treat promyelocytic leukemia [1]. Arsenic presence in various rocks and sediments is likely a result of weathered igneous rocks derived from volcanic exhalations and hot springs [2]. Arsenic concentrations in volcanic rocks were determined to be 3.5 ppm, whereas granites from Minnesota measured 1.0 ppm arsenic, on average. Arsenic in combination with elements including oxygen, chlorine, and sulfur are commonly referred to as inorganic arsenic (iAs), and they are the predominant form of environmental arsenic [3]. Arsenic is anthropogenically released into the environment due to coal burning, mining, and automobile emissions [3]. Arsenic was also used in industrial settings, primarily as a wood preservative in the form of copper chromated arsenate (CCA), commonly referred to as “pressure-treated” wood [3]. CCA was phased out of production in the United States in 2003; however, CCA-containing wood remains in use [3]. Tobacco also contains arsenic, which is absorbed from contaminated soil or soil containing naturally-derived traces of arsenic [3]. In addition, tobacco growers use pesticides containing arsenic [4]. Due to its presence in naturally- and anthropogenically-derived sources and its physiochemical properties, arsenic is capable of entering both surface and ground waters and, eventually, drinking water sources. Estimates of arsenic concentrations in rivers and lakes were determined to be as high as 1100 ppm [5]. Furthermore, agricultural products may contain arsenic due to the use of contaminated water in irrigation processes [6]. Altogether, there are many exposure pathways for arsenic, which is a particular problem considering the health effects that often accompany exposure.

Acute arsenic poisoning has been associated with abdominal pain, vomiting, diarrhea, and nausea [7]. Chronic oral exposure to arsenic causes the formation of darkened patches and small skin lesions [3]. From epidemiological studies, arsenic exposure has been shown to cause black foot disease, which is a unique peripheral vascular disease originally identified in the endemic area along the southwest coast of Taiwan [8][9]. In fact, individuals with higher arsenic exposure and lower capacity to metabolize arsenic were found to have a higher risk of developing black foot disease [8]. Moreover, epidemiological evidence shows that arsenic exposure through inhalation or drinking water causes lung, skin, and urinary bladder cancers and, as such, arsenic is classified as a Group I carcinogen by the World Health Organization (WHO) [10]. However, arsenic is not a classical carcinogen because it does not form DNA adducts or cause mutations in DNA except at unrealistically high doses, but it does induce mitotic arrest, genomic instability, and chromosomal aberrations [11][12]. Arsenic-induced carcinogenesis has also been suggested to occur by epigenetic mechanisms [13]. In addition, arsenic partially exerts its toxicity by inactivating over 200 enzymes, predominantly involved in DNA metabolism and repair [7]. Based on both human exposure potential and associated health risks, the WHO established a drinking water standard of 10 μg/L [4].

Exposure to arsenic in drinking water represents the greatest exposure pathway in Chile, Argentina, China, Mexico, Bangladesh, and the United States [4]. The first major incident of endemic disease caused by arsenic in drinking water was reported in the 1920s in Cordoba Province of Argentina [14]. In Taiwan, exposure to arsenic from drinking water was discovered in the late 1950s-early 1960s [9]. In India, arsenic poisoning was documented for the first time in 1983 when a patient presented with severe skin lesions [15]. Worldwide, as many as 140 million people are thought to be exposed to high levels of arsenic in drinking water [16]. Arsenic contamination of groundwater is a particular problem in Bangladesh and has been referred to as the largest mass poisoning of a population [15]. Out of 125 million people, between 35 and 77 million are at risk of consuming water with arsenic exceeding 10 µg/L [15][17]. In comparison, it has been estimated that 0.5–2.0, 2.0, and >3.0 million people are exposed to >10 µg/L arsenic in drinking water in Argentina, China, and in Vietnam and the United States, respectively [17]. Levels of arsenic in drinking water in Bangladesh, however, often exceed 50 µg/L, and maximum detected levels reaching 1000 µg/L and higher have been documented [18][19]. Similarly, a community of 6000 residents in the Andes Mountains in Argentina had arsenic contamination in their drinking water at levels as high as 290 µg/L [20].

In order to overcome arsenic exposure, humans have developed metabolic processes that allow for efficient excretion of arsenic in order to avoid high body burdens and systemic toxicity. The particular mechanism of arsenic metabolism and final chemical speciation of arsenic following metabolism are important considerations for its cumulative toxicity.

2. Arsenic Metabolism, Excretion, and Toxicity

Arsenic is one of the few metalloids that is metabolized in vivo. In humans and rodents, ingested arsenic compounds are detoxified and excreted following methylation. Detoxification and biotransformation pathways for iAs have only been proposed via methylated derivatives, first by the formation of monomethylarsonic acid (MMA) and then dimethylarsinic acid (DMA) [21]. Arsenic is not always fully methylated, and a portion of it is excreted as iAs and MMA. Arsenic methyltransferase is the enzyme responsible for metabolism and biotransformation of inorganic arsenic, and s-adenosylmethionine (SAM) is used as the methyl group donor [22].

Biotransformation to a methylated arsenic species to facilitate the removal of arsenic from the body was originally discovered in experiments with fungi [23]. In murine models, however, arsenic exposure via drinking water was successfully shown to produce elevated levels of methylated arsenic in urine [24]. Animals differ in the ratios of methylated arsenic species produced, and in their ability to methylate iAs [25]. Chimpanzee and marmosets do not methylate iAs, whereas hamsters and rabbits are enzymatically capable of converting iAs to DMA. Dogs and mice excrete approximately 81% and 71% DMA, respectively. Primary rat hepatocytes have been shown to methylate arsenic better than primary human cell lines, e.g., hepatocytes and keratinocytes [26], which has been suggested as one of the reasons why arsenic is less hazardous to animals. Elaborate studies on urinary excretion of arsenic metabolites were performed after repeated ingestion of iAs in humans. Upon single ingestion of iAs, 75% was shown to be excreted as methylated iAs, of which one-third was MMA and two-thirds DMA [27] .In order to develop an exposure history in industrial workers, the same authors also analyzed the excretion of arsenic metabolites in subjects after the ingestion of arsenic in graded doses for several days. About 60% of the ingested arsenic was excreted every day [28], and the composition of arsenic species excreted was similar to previous analyses (vide supra). Additionally, Vahter (1999) showed that the metabolic byproducts of arsenic are produced in different ratios in humans compared to most mammals and that humans excrete more MMA [25].

Although the mechanisms for the more rapid excretion of methylated arsenic compared to inorganic arsenic is not usually discussed, we hypothesize that this is due to the degree of protein-bound arsenic [29]. The same principle applies to the half-life of most drugs with those that are tightly protein bound having longer half-lives than those loosely bound. Thus, arsenite which is the major species in vivo, would have three protein binding sites which makes it more difficult to dissociate from a protein during passage through the kidney. In contrast, MMA and DMA would only have two and one protein binding sites, respectively, and would dissociate from protein more rapidly with a lower number of binding sites when passing through the kidney.

MMA is often considered more toxic than DMA, and higher body burdens of MMA have been suggested to be the reason why humans show toxic effects such as skin pigmentation and certain cancers [21]. However, complete methylation to the ultimate form As species is likely the most favorable outcome, despite the highly reactive and toxic intermediate metabolites. Yokohira et al. (2010) showed that more severe lesions were observed in urinary bladder epithelial cells in *AS3MT* knockout mice compared to wild-type mice [30]. In addition, severe systemic toxicity and urinary bladder cytotoxicity and regenerative hyperplasia were induced in *AS3MT* knockout mice [30]. Mechanistically, MMA^{III} and DMA^{III} were both shown to be more toxic and reactive compared to iAs^{III}, and were shown to cause apoptosis via

oxidative stress accompanied by loss of mitochondrial membrane potential and release of cytochrome C [31][32]. Therefore, it is likely that mammals have evolved to metabolize inorganic arsenic to its relatively non-cytotoxic pentavalent forms, but encounter high cytotoxicity due to intermediate metabolites, namely MMA^{III} and DMA^{III}.

The cytotoxicity and genotoxicity of inorganic arsenic and its methylated species including methyloxoarsine ($CH_3As^{III}O$), iododimethylarsine ($CH_3As^{III}I$), monomethylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), monomethylarsonous acid (MMA^{III}), and dimethylarsinous acid (DMA^{III}) differ significantly [33]. For instance, iAs^{III} and iAs^{V} produced concentration-related linear increases in DNA damage as assessed by the single-cell gel assay (i.e., comet assay) using human peripheral blood lymphocytes, but were not significantly different from each other [33]. MMA^{III} and DMA^{III}, on the other hand, were reported to be 54 and 77 times more potent than iAs^{V} or iAs^{III}, respectively, and DMA^{III} was 270 and 386 times more potent than iAs^{V} or iAs^{III}, respectively [33]. MMA^{V} and DMA^{V} were inactive and unable to damage DNA at high concentrations, however. Neither iAs^{III}, iAs^{V}, nor methylated pentavalent arsenic species produced significant nicking, strand breaks, or alkali labile lesions in DNA as assessed by either DNA nick assay compared to methylated trivalent As species [33]. While both trivalent methylated species MMA^{III} and DMA^{III} did show DNA damage in the DNA nick assay, this only occurred at abnormally high concentrations [33]. In agreement with other studies, indirect genotoxicity has frequently been observed with arsenic species and is more likely to occur than direct interaction with DNA at realistic concentrations [12][32][33][34]. Therefore, direct genotoxicity and mutagenesis resulting from arsenic species only occur at high concentrations, as exemplified by Klein et al. (2007), and an indirect mode of genotoxicity resulting from exposure to arsenic is more realistic [12].

Moe et al. (2016) compared the cytotoxicities of iAs^{III} and iAs^{V} in human urinary bladder carcinoma T24 cells and human lung adenocarcinoma A549 cells, and iAs^{III} was reported to have a significantly lower IC_{50} than IAs^{V}, which represents the concentration of arsenic species that results in a 50% reduction in cell index compared to unexposed cells and reflects cell death [21]. Moreover, the cytotoxicities of 14 additional arsenic species were determined. The reported cytotoxicities of the arsenic species tested were as follows: phenylarsine oxide, PAO^{III}; >methylarsine oxide, MAO^{III}; >MMA^{III}; >DMA^{III}; >dimethylarsinic glutathione, $DMAG^{III}$; >dimethylmonothioarsinate, $DMMTA^{V}$; >As^{III}; > monomethyltrithioarsonate, $MMTTA^{V}$; >As^{V}; >dimethyldithioarsinate, $DMDTA^{V}$; >DMA^{V}; >MMA^{V}; >roxarsone, Rox; >and p-arsanilic acid, >p-ASA [21]. Notably, all trivalent As species were the most cytotoxic in both cell lines tested, with the exception of $DMMTA^{V}$. Rox, a common pesticide used in the poultry industry, and p-ASA were the least cytotoxic as their IC_{50} values exceeded 9 and 6 mM in A549 and T24 cells, respectively [21][35]. The carcinogenicity of methylated As species was also tested in liver and prostate cell lines, and MMA^{III} was determined to cause an increase in invasiveness and colony formation in soft agar—a measure of anchorage-independent growth and hallmark of cancer [34]. While there may be slight discrepancies in the reported cytotoxicities between MMA^{III} and DMA^{III}, inorganic and organic trivalent arsenic species are certainly more cytotoxic compared to pentavalent forms [21].

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