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Arsenite and monomethylarsonous acid generate oxidative stress response in human bladder cell culture

K.E. Eblin [a,*], M.E. Bowen [a], D.W. Cromey [b], T.G. Bredfeldt [a], E.A. Mash [c], S.S. Lau [a], A.J. Gandolfi [a]

[a] Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, 1703 E. Mabel, PO Box 210207, Tucson, AZ 85721, USA
[b] Department of Cell Biology and Anatomy, University of Arizona, Tucson, AZ 85721, USA
[c] Department of Chemistry, University of Arizona, Tucson, AZ 85721, USA

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Abstract

Arsenicals have commonly been seen to induce reactive oxygen species (ROS) which can lead to DNA damage and oxidative stress. At low levels, arsenicals still induce the formation of ROS, leading to DNA damage and protein alterations. UROtsa cells, an immortalized human urothelial cell line, were used to study the effects of arsenicals on the human bladder, a site of arsenical bioconcentration and carcinogenesis. Biotransformation of As(III) by UROtsa cells has been shown to produce methylated species, namely monomethylarsonous acid [MMA(III)], which has been shown to be 20 times more cytotoxic. Confocal fluorescence images of UROtsa cells treated with arsenicals and the ROS sensing probe, DCFDA, showed an increase of intracellular ROS within five min after 1 μM and 10 μM As(III) treatments. In contrast, 50 and 500 nM MMA(III) required pretreatment for 30 min before inducing ROS. The increase in ROS was ameliorated by preincubation with either SOD or catalase. An interesting aspect of these ROS detection studies is the noticeable difference between concentrations of As(III) and MMA(III) used, further supporting the increased cytotoxicity of MMA(III), as well as the increased amount of time required for MMA(III) to cause oxidative stress. These arsenical-induced ROS produced oxidative DNA damage as evidenced by an increase in 8-hydroxyl-2'-deoxyguanosine (8-oxo-dG) with either 50 nM or 5 μM MMA(III) exposure. These findings provide support that MMA(III) cause a genotoxic response upon generation of ROS. Both As(III) and MMA(III) were also able to induce Hsp70 and MT protein levels above control, showing that the cells recognize the ROS and respond. As(III) rapidly induces the formation of ROS, possibly through it oxidation to As(V) and further metabolism to MMA(III)/(V). These studies provide evidence for a different mechanism of MMA(III) toxicity, one that MMA(III) first interacts with cellular components before an ROS response is generated, taking longer to produce the effect, but with more substantial harm to the cell.

Keywords: As (III); MMA(III); Oxidative stress; ROS; DCFDA; 8-oxo-dG

Introduction

This study was undertaken to elucidate the mechanism of arsenical-induced cellular/DNA damage and to determine if low-level arsenicals can generate an oxidative stress response. The levels of arsenicals [1 μM As (III) or approximately 7.5 ppb] used in this study fall within the range seen in the drinking water in the United States and throughout the world (Meza et al., 2004). In addition, the lower levels of MMA (III) used in this study fall within the range seen in human urine in the United States and Mexico [50 nM MMA (III)] (Mandal et al., 2001, 2004). Arsenic, a class A carcinogen, is an environmental pollutant found in soil, air, food, and in particular, water. It is considered a potent human hazard because of its ability to induce carcinogenesis. Arsenic has been found to form cancers in the lung, liver, kidney, skin, and bladder (Tseng, 2004; Simeonova and Luster, 2003). The mechanisms by which arsenic induces these diseases and carcinogenesis are poorly understood, but several possibilities

* Corresponding author.
E-mail address: eblin@pharmacy.arizona.edu (K.E. Eblin).

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have been proposed such as the induction of chromosomal abnormality, the generation of oxidative stress, the promotion of tumorigenesis, and the formation of DNA adducts and/or damage. Oxidative stress and reactive oxygen species (ROS) generated as a consequence of arsenical exposure have been linked to alterations in cell signaling, apoptosis, and increase in cytokine production leading to inflammation, which in turn leads to more ROS, and mutagenesis, which could contribute to the pathogenesis of arsenical-induced diseases (Yamanaka et al., 2004).

Arsenicals have commonly been seen to induce reactive oxygen species (ROS) and free radicals leading to an imbalance between the production of these molecules and the cellular antioxidant defenses (Shi et al., 2004a, 2004b). This imbalance can lead to tissue injury and the disruption of many physiological functions (Tseng, 2004). It has been previously shown that ROS are directly involved in oxidative damage seen in lipids, proteins, and DNA in cells exposed to arsenicals, even sometimes leading to cellular apoptosis (Shi et al., 2004a, 2004b; Kitchin and Ahmad, 2003). The production of superoxide radical can lead to DNA damage, hydroxyl radicals can lead to genotoxicity, and general oxidative stress can also lead to cellular apoptosis (Shi et al., 2004b). Another cause of oxidative stress is the methylated arsenic metabolites, which have been shown to cause DNA strand breaks and chromosomal mutations, particularly from the trivalent arsenicals (Liu et al., 2001; Hei and Filipic, 2004).

The bladder is a target organ in relation to both inorganic and methylated arsenical toxicity as it is the primary route of arsenic excretion and also is systemically exposed to arsenicals (Tapio and Grosche, 2006). These methylated arsenicals have also been shown to be up to 20 times more cytotoxic than the inorganic arsenicals, leading to increased stress to the cell (Styblo et al., 2000; Petrick et al., 2000). The methylated arsenicals, such as monomethylarsonous acid [MMA (III)] (Aposhian, 1997), are excreted at much higher levels in human urine than in any other species (Aposhian et al., 2000; Vahter, 1994). In addition to the presence of these arsenic metabolites in urine, MMA and DMA tissue levels have been shown to be at substantial levels, increasing the exposure of the bladder to these toxic substances (Kitchin and Ahmad, 2003). In human urothelial cells, known as UROtsa, arsenite has been shown to be biotransformed to MMA (III) at a rate of 3–5% (Bredfeldt et al., 2004). Based on this information, both As (III) and MMA (III) are of particular interest to study in relation to the bladder as arsenicals have been seen to induce bladder cancer in epidemiological studies.

To study the generation of ROS in the bladder, the human urothelial cell line, UROtsa (Petzoldt et al., 1995), was chosen. After immortalization via a temperature-sensitive SV40 large T-antigen gene construct, these cells maintained the expected structural characteristics of the human urothelium when grown in serum-free conditions (Rossi et al., 2001). UROtsa cells do not exhibit anchorage-independent growth or tumorigenicity in nude mice (Petzoldt et al., 1995; Sens et al., 2004).

To visualize the formation of ROS in live UROtsa cells, the fluorogenic ester, 5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate (CM-H_2DCFDA) was used. CM-H_2DCFDA has been widely used, as it is cell permeant and fluoresces in the presence of ROS (Shi et al., 2004a, 2004b; Liu et al., 2001). An important marker of oxidative stress is that of DNA oxidation by ROS, forming 8-hydroxyl-2′-deoxyguanosine (8-oxo-dG). The induction of stress proteins such as heat shock protein 70 (Hsp70) and various metallothionein isoforms (MT) is another marker of arsenical-induced toxicity and oxidative stress. This induction of stress proteins shows that the cells are compensating for the insult caused by the arsenical. UROtsa cells have normal basal levels of MT and Hsp70 when untreated with arsenicals making these two ideal markers for cellular stress to the UROtsa cell line. The heat shock protein system is also useful for study as it is widely accepted that it plays a major role in the cell's ability to protect against and recover from a toxicant insult as well as it being a rapidly inducible system useful for acute studies (Rossi et al., 2002).

As (III) had been previously shown to stimulate the formation of ROS, but the comparison to MMA (III) has never been performed, and the mechanism by which MMA (III) is 20 times more cytotoxic is still unclear (Aposhian, 1997; Aposhian et al., 2000; Petrick et al., 2000). This study focuses on the short-term effects of arsenicals via the production of ROS and oxidative stress to determine if there are differences in As (III)- and MMA (III) induced-cellular damage and to see if these differences occur at biologically relevant concentrations.

Materials and methods

Chemicals

Arsenicals. $NaAsO_2$ (>98% pure) was purchased from Sigma Aldrich (St. Louis, MO). MMA (III) was synthesized by the Synthetic Facility Core of the Southwest Environmental Health Sciences Center [University of Arizona] (Millar et al., 1960). MMA (III) is stable in solution in H_2O for up to 4 months as determined by ICP-MS (data not shown).

Cell culture supplements and media. Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium, and antibiotic–antimycotic were purchased from Invitrogen (Carlsbad, California).

Cells. UROtsa cells were a generous gift from Drs. Donald and Maryann Sens (University of North Dakota). Cell culture conditions were derived from those described by Bredfeldt et al. (2004). Cells were grown on polystyrene (100×20 mm) plates using DMEM enriched with 5% FBS and 1% antibiotic–antimycotic at 37 °C in 5% CO_2. Prior to experimentation, cells were fed a serum-free growth medium made up of 1:1 mixture of DMEM and Ham's F-12 supplemented with insulin (5 μg/ml), hydrocortisone (36 ng/ml), and epidermal growth factor (10 ng/ml) every 2 days. At confluence, cells were removed from polystyrene using 0.25% trypsin/EDTA (1 mM) and subcultured at a ratio of 1:3. Cells were allowed to become confluent before experiments were conducted.

For confocal microscopy, the cells were plated on Delta T dishes (Bioptech, Butler, PA) at 500,000 cells per plate and allowed to reach 90% confluency before experimentation.

Biotransformation assay. Biotransformation studies were used to detect if UROtsa cells would further biotransform MMA (III). Cells were exposed for 24-h intervals with 50 nM MMA (III). After MMA (III) exposure, both cell culture media and lysates were analyzed for arsenicals (Bredfeldt et al., 2004). Filtrates will be separated and detected by HPLC (Agilent 1500) coupled to ICP-MS (Agilent 7500a). The limit of detection for each analyte was 0.5 ppb.