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Comparative cytotoxicity of fourteen trivalent and pentavalent arsenic species determined using real-time cell sensing☆

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ABSTRACT

The occurrence of a large number of arsenic species in the environment and in biological systems makes it important to compare the relative toxicity of the diverse arsenic species. Toxicity of arsenic species has been examined in various cell lines using different assays, making comparison difficult. We report real-time cell sensing of two human cell lines to examine cytotoxicity of fourteen arsenic species: arsenite (As^{III}), monomethylarsonous acid (MMA^{III}) originated from the oxide and iodide forms, dimethylarsinous acid (DMA^{III}), dimethylarsinic glutathione ($DMAG^{III}$), phenylarsine oxide (PAO^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), monomethyltrithioarsonate ($MMTTA^{V}$), dimethylmonothioarsinate ($DMMTA^{V}$), dimethyldithioarsinate ($DMDTA^{V}$), 3-nitro-4-hydroxyphenylarsonic acid (Roxarsone, Rox) and 4-aminobenzenearsenic acid (p-arsanilic acid, p-ASA). Cellular responses were measured in real-time for 72 hr in human lung (A549) and bladder (T24) cells. IC_{50} values for the arsenicals were determined continually over the exposure time, giving rise to IC_{50} histograms as unique cell response profiles. Arsenic accumulation and speciation were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS). On the basis of the 24-hr IC_{50} values, the relative cytotoxicity of the tested arsenicals was in the following decreasing order: $PAO^{III} \gg MMA^{III} \geq DMA^{III} \geq DMAG^{III} \approx DMMTA^{V} > As^{III} \gg MMTTA^{V} > DMDTA^{V} > As^{V} \gg DMA^{V} > MMA^{V} > Rox \geq$ p-ASA. Step-wise shapes of cell response profiles for DMA^{III}, $DMAG^{III}$, and $DMMTA^{V}$ coincided with the conversion of these arsenicals to the less toxic, pentavalent DMA^{V}. Dynamic monitoring of the real-time cellular responses to arsenicals provided useful information for comparison of the relative cytotoxicity of arsenicals.

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Introduction

Arsenic occurs naturally throughout the geosphere and is ubiquitous in the environment (Cullen and Reimer, 1989). Arsenic contamination of groundwater that serves as human drinking water sources is a serious public health concern (NRC, 1999). Chronic consumption of inorganic arsenic at elevated concentrations is a known cause of skin, bladder, and lung cancers (NRC, 2001; IARC, 2012; Cohen et al., 2016), and has also been associated with kidney, liver, and prostate cancers (IARC, 2012) as well as several non-carcinogenic ailments including diabetes, reproductive, cardiovascular, and neurological diseases (Schuhmacher-Wolz et al., 2009; Hughes et al., 2011; Maull et al., 2012; Naujokas et al., 2013). In humans, inorganic arsenic is enzymatically biotransformed to several methylated metabolites. This pathway of inorganic arsenic metabolism is generally accepted to follow: inorganic arsenate [As^{V}] → inorganic arsenite [As^{III}] → monomethylarsonic acid [MMA^{V}] → monomethylarsonous acid [MMA^{III}] → dimethylarsinic acid [DMA^{V}] → dimethylarsinous acid [DMA^{III}] (Challenger, 1945; Cullen et al., 1989; Le et al., 2000; Styblo et al., 2002; Vahter, 2002; Thomas et al., 2001, 2004, 2007; Cullen, 2014). Alternative pathways postulating glutathione- or protein-conjugated intermediates have also been proposed (Hayakawa et al., 2005; Naranmandura et al., 2006; Dheeman et al., 2014), although chemical basis has been questioned (Cullen, 2014).

Arsenic cytotoxicity is dependent on its oxidation state and chemical structure (speciation). In general, trivalent arsenicals are more cytotoxic than pentavalent species, and the methylated trivalent arsenicals, MMA^{III} and DMA^{III}, are more cytotoxic than the inorganic arsenicals, As^{III} and As^{V}, which are more cytotoxic than the methylated pentavalent arsenicals, MMA^{V} and DMA^{V} (Styblo et al., 2000; Petrick et al., 2000; Dopp et al., 2004; Nascimento et al., 2008; Charoensuk et al., 2009; Naranmandura et al., 2011). Historically, the focus of arsenic toxicity studies has been the examination of the oxygenated metabolites of inorganic arsenic. However, as analytical techniques have improved to increase sensitivity and specificity, several thiolated arsenic metabolites have been identified (Hansen et al., 2004; Wang et al., 2015; Chen et al., 2016; Sun et al., 2016). A class of thiol-containing arsenicals that were first identified as metabolites in seaweed-fed sheep (Hansen et al., 2004), are the pentavalent sulfur-containing arsenic species, such as dimethylmonothioarsinate [$DMMTA^{V}$], dimethyldithioarsinate [$DMDTA^{V}$], and monomethylmonothioarsonate [$MMMTA^{V}$]. These thio-arsenicals have been detected in human or animal urine as metabolites of inorganic arsenic (Hansen et al., 2004; Naranmandura et al., 2007b; Raml et al., 2007; Naranmandura et al., 2013; Chen et al., 2016), and are believed to be formed from reactions between oxygenated arsenicals and hydrogen sulfide (Wang et al., 2015). Monomethyltrithioarsonate [$MMTTA^{V}$] is another thiol-containing pentavalent metabolite, but has only been found as a metabolite of anaerobic microbiota *in vitro* (Pinyayev et al., 2011). Recent cytotoxicity analysis of these newly identified thiolated pentavalent arsenicals suggests that thiol conjugation can modulate arsenic toxicity, prompting inclusion of thiolated metabolites in this study. $DMMTA^{V}$ has been found to be as toxic as the trivalent species, As^{III} and DMA^{III}, in human cancer cell lines (Naranmandura et al., 2007a; Naranmandura et al., 2009). The trivalent glutathione conjugated arsenical, dimethylarsinic glutathione [$DMAG^{III}$], is suspected to play a key role in the transport of methylated arsenic species from the liver to the blood stream (Percy and Gailer, 2008). Reported IC_{50} values for $DMAG^{III}$ are equal to or less than those of As^{III} (Styblo et al., 2000; Vega et al., 2001).

While inorganic arsenic and its metabolites are often considered the most important from a human health perspective, other organoarsenic species have become topics of recent research interest. Two pentavalent phenyl arsenic species used in poultry industry are 3-nitro-4-hydroxyphenylarsonic acid (Roxarsone, Rox) and 4-aminobenzenearsenic acid (p-arsanilic acid, p-ASA). As poultry feed additives, Rox and p-ASA not only improve feed efficiency, allowing for faster weight gain, but also help control intestinal bacteria and parasites (Jones, 2007; Chen and Huang, 2012; Nachman et al., 2013). Both arsenicals have been phased out of use in the European Union and the United States; however, they are still uses in many other countries (Kazi et al., 2013; Yao et al., 2013; Mafla et al., 2015; Mangalgiri et al., 2015; Wang and Cheng, 2015). Although several studies have shown that these arsenicals can accumulate in the tissue and organs of livestock (Aschbacher and Feil, 1991; Desheng and Niya, 2006; Nachman et al., 2013; Peng et al., 2014; Liu et al., 2015; Liu et al., 2016), little is known about the cytotoxicity of these pentavalent arsenic species to human cells. Another arsenic species that is used in laboratory research as a known inhibitor in various biochemical reactions to elucidate toxicity mechanisms is phenylarsine oxide [PAO^{III}]. This trivalent organoarsenic species is not naturally-occurring, but it is found in the environment at sites contaminated with chemical warfare agents, as it is a degradation product of the chemical warfare agent, diphenylarsine dichloride (also known as Pfiffikus) (Leermakers et al., 2006). Studies have shown PAO^{III} to be a potent cytotoxicant (Charoensuk et al., 2009).

Extensive data are available surrounding the cytotoxicity of individual arsenicals. However, these data have been obtained using various assays on different cell lines. The species-dependent cytotoxicity and variations in different assays make it difficult to compare the relative cytotoxicity of different arsenic species. In addition, some arsenicals have therapeutic uses, as with the treatment of acute promyelocytic leukemia with arsenic trioxide (As_2O_3) (Shen et al., 1997; Wang et al., 2004; Chen et al., 2015) and refractory solid tumors with $DMAG^{III}$ (alternate names: S-dimethylarsino-glutathione, ZIO-101, and darinaparsin). Understanding the relative cytotoxicity of various arsenic species may direct its exploitation for further therapeutic investigation.

Real-time cell-electronic sensing analysis is an impedance-based detection technique that can simultaneously perform 96 *in vitro* tests of cytotoxicity. Because the real-time cell sensing technique is label-free and dye-free, it is less invasive than traditional colorimetric cytotoxicity assays. It provides continuous monitoring, revealing more dynamic and complete cytotoxic response information, and it has been demonstrated in chemical cytotoxicity testing (Solly et al., 2004; Xing et al., 2005; Boyd et al., 2008; Moe et al., 2016). It is also one of the cell-based *in vitro* assay technologies implemented in the United States Environmental Protection Agency ToxCast program to prioritize the vast number of environmental chemicals, many of which are already under heavy commercial use, for

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