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Trivalent arsenicals induce lipid peroxidation, protein carbonylation, and oxidative DNA damage in human urothelial cells

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Abstract

Drinking arsenic-contaminated water is associated with an increased risk of bladder cancer. Arsenate (iAs^V), arsenite (iAs^{III}), monomethylarsonous acid (MMA^{III}), monomethylarsonic acid (MMA^V), dimethylarsinous acid (DMA^{III}), and dimethylarsinic acid (DMA^V) have all been detected in the urine of people who drink arsenic-contaminated water. The aim of this research was to investigate which of these arsenicals are more hazardous to human urothelial cells. The results indicate that iAs^{III}, MMA^{III}, and DMA^{III} were more potent in inducing cytotoxicity, lipid peroxidation, protein carbonylation, oxidative DNA damage, nitric oxide, superoxide, hydrogen peroxide, and cellular free iron than MMA^V, DMA^V, and iAs^V in human urothelial carcinoma and transformed cells. However, the results did not show convincingly that the trivalent arsenicals were more potent than pentavalent arsenicals in decreasing the intracellular contents of total thiol, protein thiol, and reduced glutathione. Induction of oxidative DNA damage was observed with 0.2 μM of iAs^{III}, MMA^{III}, or DMA^{III} as early as 1 h. Because of its high oxidative damage, higher proportion in urine, and lower cytotoxicity, DMA^{III} may be the most hazardous arsenical to human urothelial cells.

Keywords: Comet assay; Endonuclease III; Formamidopyrimidine-DNA glycosylase; HEK 293 cells; SV-HUC-1 cells

1. Introduction

Arsenic is ranked first in priority among a listing of the top 20 hazardous substances by the Agency for Toxic Substances and Disease, Atlanta, and bladder cancer is considered to be the greatest risk for long-term arsenic exposure [1]. The relative risks of developing transitional cell carcinoma were estimated to be 8.2 and 15.3 for drinking water containing arsenic concentrations of 50.1–100 and >100 μg/L, respectively [2]. The association between arsenic exposure from contaminated drinking water and an increased incidence of urinary bladder cancer has been reported in epidemiological studies conducted in Argentina [3], Chile [4], Japan [5], Taiwan [6], and USA [7]. The arsenicals in drinking water are mainly in inorganic pentavalent and trivalent forms. In the human body, pentavalent arsenate is reduced to trivalent arsenite, and then methylated to monomethylarsonic acid (MMA^V), monomethylarsonous acid (MMA^{III}), dimethylarsinic acid (DMA^V), and dimethylarsinous acid (DMA^{III}) by the alternate reduction of arsenic from pentavalent to trivalent forms and the addition of methyl groups [8]. The methylation pathway has long been

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considered to be a method of detoxification, but recent studies have indicated that MMA^{III} and DMA^{III} are more toxic than inorganic trivalent arsenic [9,10]. Arsenate (iAs^{V}), arsenite (iAs^{III}), MMA^{III}, MMA^{V}, DMA^{III}, and DMA^{V} are all detectable in the urine of people who drink arsenic-contaminated water [11].

Earlier works indicated that very high concentrations of arsenite are required to induce DNA strand breaks [12,13], however, by incorporating enzyme digestion, iAs^{III}, MMA^{III}, and DMA^{III} have been shown to induce oxidative DNA damage at submicromolar concentrations [14–16]. These data are consistent with reports that arsenic exposure increases the frequency of chromosomal aberrations [17] and sister chromatid exchanges [18] in arsenic-exposed humans. Several independent studies indicated that increased levels of micronucleated cells in exfoliated bladder cells are associated with elevated concentrations of arsenic in drinking water [19–22]. Bladder tumor patients with higher levels of arsenic exposure showed higher levels of chromosomal instability, as analyzed by comparative genomic hybridization [23]. Another study demonstrated that the level of micronucleated cells decreased if drinking water contaminated with high concentrations of arsenic was replaced with that containing low concentrations of arsenic [24]. These data provide strong arguments for DNA damage as a mechanism for arsenic carcinogenesis.

Arsenic is generally recognized as a thiol-reacting agent, and is assumed to express its toxicity by reacting to the thiol groups of cellular molecules [25]. The protective effects of thiols and dithiols against the toxic effects of arsenic suggest that arsenic toxicity results from the formation of reversible bonds with the thiol groups of regulatory proteins. The whole-blood non-protein sulfhydryl level in arsenic-exposed subjects was 60% that of the controls [26]. Glutathione reduces arsenate to arsenite which subsequently forms a $(GS)_3As^{III}$ complex [27]. Glutathione is also involved in the reductive metabolism and methylation of arsenic [28]. Arsenite is known to inhibit pyruvate dehydrogenase via covalent binding to critical thiols that are part of the lipoamide moieties in this multienzyme complex. However, recent evidence has shown that arsenite inhibits pyruvate dehydrogenase activity by oxidation or nitration rather than by reacting to thiols [29].

In recent years, evidence has been accumulated to show that reactive oxygen and nitrogen species are involved in arsenic toxicity [30–32]. Sufficient evidence has shown that arsenic can induce oxidative DNA damage [30–33]. Toxic reactive oxygen species including superoxide and hydroxyl radicals, and hydrogen peroxide, generated from normal cellular respiration and aerobic metabolism or exogenous oxidants, cause cellular damage by oxidizing nucleic acids, proteins, and membrane lipids [34]. However, relatively few studies have dealt with the effects of arsenic on lipids and proteins. The mediation of lipid peroxides by reactive oxygen species is of critical importance because they participate in chain reactions that amplify damage to biomolecules. Both lipid peroxides and their breakdown products, such as malonaldehyde and 4-hydroxy-2-nonenal, directly and indirectly affect many functions integral to cellular and organ homeostasis. As a result, increased membrane lipid peroxidation may evoke immune and inflammatory responses [35], activate gene expression [36] and cell proliferation [37], or initiate apoptosis [38]. Reactive oxygen species also cause a number of nonenzymatic modifications to proteins, including carbonylation, *o*-tyrosine, chlorotyrosine, nitrotyrosine, and dityrosine [39]. Perhaps most significantly, carbonylation of a protein reduces its activity. Consequently, cells that have large concentrations of protein carbonyls may be expected to have impaired function.

The aims of this investigation were to find out which arsenic species are more toxic to human urothelial cells and what the possible mechanism is.

2. Materials and methods

2.1. Cell culture

The human urothelial carcinoma cell lines, BFTC905 [40] and NTUB1 cells [41], were grown in RPMI 1640 medium. The HEK 293 cell line is derived from adenovirus-transformed human embryonic kidney epithelial cells. The SV-HUC-1 cell line is a SV40 large T-transformed human urothelial cell line. SV-HUC-1 and HEK 293 cells were cultured in F-12 and DMEM media, respectively. All growth media were supplemented with 10% fetal calf serum, penicillin (100 units/mL), streptomycin (100 μg/mL), and 0.03% glutamine. Cultures were maintained at 37 °C in a water-saturated atmosphere containing 5% CO_2.

2.2. Chemicals

Sodium arsenite (>98.5%, iAs^{III}), disodium hydrogen arsenate (>98.5%, iAs^{V}), and dimethylarsinic acid sodium trihydrate (>97%, DMA^{V}) were from Merck (Darmstadt, Germany). Monomethylarsonic acid (>99%, MMA^{V}) was from Chemical Services (West Chester, PA). The source of MMA^{III} was the solid oxide (CH_3AsO), and that of DMA^{III} was the iodide [$(CH_3)_2AsI$]. The precursors were prepared following procedures described in the literature [42,43] and were kept at −20 °C. Dilute solutions of the precursors were freshly prepared in deionized water to form $CH_3As(OH)_2$ (MMA^{III})