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Genotoxic and clastogenic effects of monohaloacetic acid drinking water disinfection by-products in primary human lymphocytes

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ABSTRACT

The haloacetic acids (HAAs) are the second-most prevalent class of drinking water disinfection by-products formed by chemical disinfectants. Previous studies have determined DNA damage and repair of HAA-induced lesions in mammalian and human cell lines; however, little is known of the genomic DNA and chromosome damage induced by these compounds in primary human cells. The aim of this study was to evaluate the genotoxic and clastogenic effects of the monoHAA disinfection by-products in primary human lymphocytes. All monoHAAs were genotoxic in primary human lymphocytes, the rank order of genotoxicity and cytotoxicity was IAA > BAA >> CAA. After 6 h of repair time, only 50% of the DNA damage (maximum decrease in DNA damage) was repaired compared to the control. This demonstrates that primary human lymphocytes are less efficient in repairing the induced damage by monoHAAs than previous studies with mammalian cell lines. In addition, the monoHAAs induced an increase in the chromosome aberration frequency as a measurement of the clastogenic effect of these compounds. These results coupled with genomic technologies in primary human cells and other mammalian non-cancerous cell lines may lead to the identification of biomarkers that may be employed in feedback loops to aid water chemists and engineers in the overall goal of producing safer drinking water.

Abbreviations: BAA, Bromoacetic acid; CA, Chromosome aberrations; CAA, Chloroacetic acid; CHO cells, Chinese hamster ovary cells; DBPs, Disinfection by-products; EMS, Ethylmethane sulfonate; FBS, Fetal bovine serum; HAA, Haloacetic acids; IAA, Iodoacetic acid; SCGE, Single cell gel electrophoresis.

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1. Introduction

During the 20th century water disinfection was an outstanding public health success to control waterborne diseases (Reynolds et al., 2008). However, during the water disinfection process, disinfection by-products (DBPs) are unintentionally formed (Richardson, 2009). Many DBPs are cytotoxic, genotoxic and teratogenic (Hunter et al., 1996; Plewa and Wagner, 2009; Plewa et al., 2002; Richardson et al., 2008). Furthermore, in epidemiological studies, DBPs demonstrate an association with increased risk of bladder (Bove et al., 2007; Goebell et al., 2004; Villanueva et al., 2004) and colorectal cancer (Rahman et al., 2010). The haloacetic acids (HAAs) are the second-most prevalent class of DBPs formed after disinfection with chlorine (Hua and Reckhow, 2007; Krasner et al., 2006). HAAs are mutagenic in *Salmonella typhimurium* (Kargalioglu et al., 2002), cytotoxic, genotoxic and mutagenic in Chinese hamster ovary (CHO) cells (Plewa et al., 2002, 2010; 2004b; Zhang et al., 2010) and genotoxic in non-transformed human FH cells (Attene-Ramos et al., 2010). Additionally, they are genotoxic but not clastogenic in the human lymphoblastoid TK6 cell line (Liviac et al., 2010) and teratogenic in mice (Hunter et al., 1996). The U.S. Environmental Protection Agency (U.S. EPA) regulates five HAAs (chloroacetic acid (CAA), dichloroacetic acid, trichloroacetic acid, bromoacetic acid (BAA), and dibromoacetic acid) to the maximum level of 60 μg/L (U.S. EPA, 2006).

The monoHAAs have a single halogen substituent and they include CAA, BAA and iodoacetic acid (IAA), with the following order of toxicity: IAA > BAA >> CAA (Attene-Ramos et al., 2010; Hunter et al., 1996; Kargalioglu et al., 2002; Plewa and Wagner, 2009; Plewa et al., 2002, 2004b; Zhang et al., 2010). These monoHAAs are alkylating agents that undergo S_N2 reactions, which highly correlate with the toxicity of these chemicals (Pals et al., 2011). Because of this direct correlation between S_N2 alkylation potential and toxic responses a hypothesis was developed that direct DNA alkylation by HAAs served as their probable genotoxic mechanism. However, other research suggests that the indirect generation of reactive oxygen species (ROS) within cells, initiated by the exposure to HAAs, causes the observed manifold toxic responses (Cemeli et al., 2006; Larson and Bull, 1992; Pals et al., 2011; Parrish et al., 1996; Plewa et al., 2004b). Previously, it was reported that HAAs induced genomic DNA damage and mutagenicity in CHO cells without exogenous cytochrome P450 activation (Plewa et al., 2010, 2004b; Zhang et al., 2010). ROS induce oxidative damage and the most common primary DNA lesions that arise are oxidized bases, single- and double-strand DNA breaks (Tudek et al., 2010).

Studies have been published on the induction of DNA damage and repair of DBP-induced lesions in mammalian and cancerous human cell lines, however, little is known on the effect of monoHAAs in primary human cultures. We hypothesized that primary human lymphocytes will display altered genotoxic and clastogenic responses after acute exposure to the monoHAAs. The objectives of this research were, 1) to determine the genotoxicity and kinetics of DNA repair induced by the monohaloacetic acids in primary human lymphocytes and 2) to determine the clastogenic effect of these drinking water disinfection by-products on these primary cells.

2. Materials and methods

2.1. Reagents

General cell culture reagents were purchased from Sigma Chemical Co. (St. Louis, MO). BAA and CAA were purchased from Fluka Chemical Co. (Buchs, Switzerland) and IAA was purchased from Sigma–Aldrich Chemical Co. (Milwaukee, WI). The DBP stock solutions were dissolved in dimethyl sulfoxide and stored at −20 °C in sealed, sterile glass vials. For the treatments, each monoHAA was dissolved in RPMI 1640 medium without fetal bovine serum (FBS). CAS numbers of chemical agents and concentrations used for all assays are presented in Table 1.

2.2. Blood samples and primary lymphocyte isolation

Primary human lymphocytes were obtained from blood samples taken from three healthy, non-smoking males, ages 26–27 years old. After the informed consent was obtained, blood was collected under protocols approved by the Ethic Committees of our institutions. Approximately 10 mL of blood were collected from each donor by venipuncture into EDTA vacutainer tubes. Blood samples were taken prior to loading every experiment and lymphocyte isolation was conducted by histopaque density gradient (Sigma Chemical Co.).

Table 1 – Drinking water disinfection by-products and positive control analyzed in human primary lymphocytes.

Chemical agent	Abbreviation	CAS number	SCGE assay[a] concentrations (μM)	DNA repair kinetics concentration (μM)	Mitotic index assay concentrations (μM)	Chromosome aberrations assay concentrations (μM)
Chloroacetic acid	CAA	79-11-8	1–2940	90	1–5880	1, 180 and 1470
Bromoacetic acid	BAA	79-08-3	4–270	34	4–4400	4, 68, and 1100
Iodoacetic acid	IAA	64-69-7	2.5–91	22	2.5–1470	2.5, 45 and 367
Ethylmethane sulfonate	EMS	62-52-0	1150–3390	2260	N.E	2260

N.E, Not evaluated.
a SCGE, single cell gel electrophoresis.