Mutation Research 770 (2014) 136–143

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Effect of drinking water disinfection by-products in human peripheral blood lymphocytes and sperm

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ARTICLE INFO

Article history:
Received 5 February 2014
Received in revised form 30 July 2014
Accepted 20 August 2014
Available online 26 August 2014

Keywords:
Bromoacetic acid (BAA)
Butylated hydroxyanisole (BHA)
CBMN (cytokinesis-block micronucleus) assay
Catalase
Chloroacetic acid (CAA)
Comet assay
Haloacetic acid (HAA)
Iodoacetic acid (IAA)
Micronuclei (MNi)

ABSTRACT

Background: Drinking water disinfection by-products (DBPs) are generated by the chemical disinfection of water and may pose hazards to public health. Two major classes of DBPs are found in finished drinking water: haloacetic acids (HAAs) and trihalomethanes (THMs). HAAs are formed following disinfection with chlorine, which reacts with iodide and bromide in the water. Previously the HAAs were shown to be cytotoxic, genotoxic, mutagenic, teratogenic and carcinogenic.
Objectives: To determine the effect of HAAs in human somatic and germ cells and whether oxidative stress is involved in genotoxic action. In the present study both somatic and germ cells have been examined as peripheral blood lymphocytes and sperm.
Methods: The effects of three HAA compounds: iodoacetic acid (IAA), bromoacetic acid (BAA) and chloroacetic acid (CAA) were investigated. After determining appropriate concentration responses, oxygen radical involvement with the antioxidants, butylated hydroxanisole (BHA) and the enzyme catalase, were investigated in the single cell gel electrophoresis (Comet) assay under alkaline conditions, >pH 13 and the micronucleus assay.
Results: In the Comet assay, BHA and catalase were able to reduce DNA damage in each cell type compared to HAA alone. In the micronucleus assay, micronuclei (MNi) were found in peripheral lymphocytes exposed to all three HAAs and catalase and BHA were in general, able to reduce MNi induction, suggesting oxygen radicals play a role in both assays.
Conclusion: These observations are of concern to public health since both human somatic and germ cells show similar genotoxic responses.

1. Introduction

The environment plays a critical role in health and disease and in general several factors, including air quality, housing and health, water and sanitation all contribute to health and disease. Water sanitation is a major health issue to address, particularly in developing countries where in some parts there is a lack of clean, safe and quality drinking water, exposing people to pathogens and disease causing organisms present in the water. In order to disinfect water, water companies and suppliers make use of disinfectants, mainly chlorine but also including alternatives: chloramines, chlorine dioxide and ozone [1–4].

Fortunately, chemical disinfectants have helped to eliminate diseases; they effectively kill harmful microorganisms found in drinking water [2,5]. These disinfectants are capable of oxidising organic matter and naturally occurring bromide and iodide found in source waters of reservoirs, lakes and rivers [1,2,5]. While disinfectants have provided a novel method as a means to clean water, their usage leads to the formation of unwanted drinking water disinfection by-products (DBPs). DBPs form as a consequence of reactions between the chemical disinfectant and organic constituents, bromide and iodide that are naturally present in water [4].

DBPs are a major public health concern. Several epidemiological studies have shown that consumption or exposure to water above the maximum containment levels of DBPs in water have been associated with problems of liver, kidney, the central nervous system and increased risks of bladder, and colorectal cancers [6–9]. DBP exposure has also been associated with spontaneous abortions,

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http://dx.doi.org/10.1016/j.mrfmmm.2014.08.003
0027-5107/

low birth weight, still births and late pregnancy preterm delivery [10–14].

These findings demonstrate an important cause for concern, suggesting the need to further study the effect of DBPs in cells that have not been reported in the current literature. The study by Cemeli et al. [15] involving iodoacetic acid and antioxidants in Chinese hamster ovary cells (CHO) mammalian cells became a starting point in our study to investigate the effect in human cells of somatic and of germ line origin. Damage to somatic and germ cells can give rise to cancer, but in germ cells damage could also produce heritable defects. There is indirect evidence that shows this is so [16–20].

To date, over 600 DBPs have now been identified, as reviewed by Richardson et al. [2]. Although it has been possible to identify a large number of DBPs, only a few of these have been further analysed for their cytotoxic and genotoxic potential and impact on health issues [1,15,21–26]. As for the regulation of DBPs, only a small number of genotoxic and cytotoxic DBPs are regulated, however many DBPs with similar consequences remain unregulated [2,23]. As with all common DBPs, HAAs can form from usage of chlorine, chloramine, chlorine dioxide and ozone; however, amongst them chlorination use produces higher levels of HAAs [2,21].

It has previously been demonstrated that the HAAs—iodoacetic acid (IAA), bromoacetic acid (BAA) and chloroacetic acid (CAA) are cytotoxic and genotoxic DBPs when analysed in the Ames test, and in the mammalian CHO cell system [1,5,23]. Cemeli et al. [15] have also shown the involvement of oxygen radicals with IAA in these cells. However, HAAs have not been found to be genotoxic in human TK6 cells in the micronucleus assay [27].

IAAs have also been reported to induce chromosomal aberrations in CHO cells [28]. Plewa et al. have shown responses amongst the HAAs for IAA, BAA and CAA when analysing HAA-induced genomic DNA damage in CHO cells. Their studies have shown that bromo-HAAs (brominated) were more cytotoxic and genotoxic than chloro-HAAs (chlorinated) analogues and that iodo-HAAs (iodinated) analogues were more cytotoxic and genotoxic when compared to both bromo-HAAs and chloro-HAAs [1,22]. These HAAs were toxic in the same order, following 24 h exposure to mice (iodo-HAAs > bromo-HAAs > chloro-HAAs) for the induction of neural tube defects in mouse embryos. Exposure also produced malpositioned and/or hypoplastic pharyngeal arches and affected heart and optic development [29].

Toxigenomic implications of HAAs were shown in studies in which mice were exposed to chloro and bromo-chloro-HAAs. Altered gene expression was found in pathways involving cell communication, cell cycle and proliferation, metabolism, signal transduction, spermatogenesis and male fertility in mice exposed to dichloroacetic acid (DCAA) [30]. Mice that were subject to bromo-chloro acetic acid (BCAA) exposure, exhibited cellular damage and altered gene expression in different pathways involving fatty acid metabolism, angiogenesis, and tissue remodelling [31]. Another study on HAA toxicogeonomics showed that BAA was able to alter transcriptome profiles for genes involved in DNA repair, including the cell cycle regulation of double stranded DNA strand breaks (dd-DSBs) [32]. In non-transformed human cell toxicogenomic analyses of the HAAs, the major pathways involved with altered gene expression were ATM, MAPK, p53, BRCA1, BRCA2, and ATR. These latter pathways highlight the involvement of DNA repair, especially the repair of double strand DNA breaks [33].

In human lymphocytes, Kogevinas et al. [34] reported damage with THMs in lymphocytes using the micronucleus assay, but not in the Comet assay. These studies involving HAAs in both *in vitro* and *in vivo* experiments, demonstrate the need for further studies to address and raise the importance of health implications posed by HAAs. The present study further determines toxic effects in *in-vitro* of HAAs in human somatic cells and for the first time also in germ cells. Samples were taken from participating consented volunteers. The Comet assay and the micronucleus assay were used to detect any genotoxic damage in these cells.

The Comet assay is a micro-electrophoretic technique for direct visualisation of DNA damage in mammalian cells [35]. The Comet assay has been recognised for its time efficiency, simplicity, sensitivity, and cost effectiveness for analysing genotoxic DNA damage in sperm and lymphocytes as shown in previous studies [36–41].

The micronucleus test was used as a cytogenetic end point in peripheral lymphocytes. The mitosis stage of the cell cycle growth can lead to the creation of small distinct extra-nuclear bodies called MNi as a consequence of chromosome damage during cell division [42–45]. Lymphocytes were scored for MNi presence in all samples since MNi equates to signs of chromosomal loss, breakage, rearrangement, non-disjunction and apoptosis [42,44,45].

2. Materials and methods

Ethical approval was obtained for performing both the Comet assay and micronucleus assay for the study of halo acetic acids (HAAs), including sample collection of human lymphocytes and sperm. All work was undertaken after approval by the University of Bradford's Subcommittee for Ethics in Research involving Human Subjects (Ethics Reference number: 0405/8).

2.1. Reagents, sample collection & study cohort

2.1.1. Both Comet and cytokinesis-block micronucleus (CBMN) assays

Slides and coverslips were obtained from VWR international. The three HAA compounds (iodoacetic, bromoacetic and chloroacetic acids), were dissolved in dimethyl sulfoxide (DMSO) and stored at −20 °C. The antioxidant, butylated hydroxyanisole (BHA) (CAS no. 25013-16-5) was dissolved in DMSO and catalase (EC 1.11.1.6) in double distilled water (ddH_2O). Both antioxidants were purchased from Sigma Chemical Co (Poole, Dorset, UK). RPMI-1640, foetal bovine serum (FBS) medium and ethidium bromide (E-8751) were purchased from Sigma Chemical Co. (Poole, Dorset, UK). RPMI 1640 medium (with L-glutamine and 25 mM Herpes), cytochalasin B (Cat No. C6762) and mitomycin C (Cat No. M0503) were purchased from Sigma-Aldrich (Poole, UK). Phythaemagglutinin (PHA) (Cat No. 10576-015), penicillin–streptomycin solution (Cat No. 15140-122 were purchased from Invitrogen Ltd, UK. The Fixogum rubber cement was purchased from Marabu, Germany.

2.1.2. Sample collection

Healthy volunteers donated blood with consent. Lymphocytes were isolated using Lymphoprep™ (Axis-Shield, Oslo) and frozen in DMSO at −20 °C and then at −80 °C. Healthy volunteers provided semen samples through masturbation after abstinence for a minimum of 3 days. Conventional semen analysis was performed using a 100 μl sample [46]. The remaining samples were flash frozen in liquid nitrogen and stored at −80 °C.

2.1.3. Study cohort

In this study, samples were used from different donors in lymphocyte cells and sperm.

2.1.4. Comet assay lymphocytes

A total of 12 blood samples were used. These blood samples were obtained from healthy donors, and lymphocytes were isolated and stored at −80 °C in foetal calf-serum (FCS) and dimethylsolfoxide (DMSO). Frozen samples were thawed and then used for experiments. Four samples were used in succession to perform the Comet assay with one HAA (*e.g.* IAA). The results from the four experiments were then combined and averaged to create a data set (Table 1a).