Toxicology and Applied Pharmacology 347 (2018) 70–78

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Hazard assessment of three haloacetic acids, as byproducts of water disinfection, in human urothelial cells

Alicia Marsà[a], Constanza Cortés[a], Alba Hernández[a,b], Ricard Marcos[a,b,*]

[a] Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[b] CIBER Epidemiología y Salud Pública, ISCIII, Spain

ARTICLE INFO

Keywords:
Haloacetic acids
Chloroacetic acid
Bromoacetic acid
Iodoacetic acid
Cell transformation
T24 cells

ABSTRACT

Disinfection by-products (DBPs) are compounds produced in the raw water disinfection processes. Although increased cancer incidence has been associated with exposure to this complex mixture, the carcinogenic potential of individual DBPs remains not well known; thus, further studies are required. Haloacetic acids (HAAs) constitute an important group among DBPs. In this study, we have assessed the *in vitro* carcinogenic potential of three HAAs namely chloro-, bromo-, and iodoacetic acids. Using a long-term (8 weeks) and sub-toxic doses exposure scenario, different *in vitro* transformation markers were evaluated using a human urothelial cell line (T24). Our results indicate that long-term exposure to low doses of HAAs did not reproduce the genotoxic effects observed in acute treatments, where oxidative DNA damage was induced. No changes in the transformation endpoints analyzed were observed, as implied by the absence of significant morphological, cell growth rate and anchorage-independent cell growth pattern modifications. Interestingly, HAA-long-term exposed cells developed resistance to oxidative stress damage, what would explain the observed differences between acute and long-term exposure conditions. Accordingly, data obtained under long-term exposure to sub-toxic doses of HAAs could be more accurate, in terms of risk assessment, than under acute exposure scenarios.

1. Introduction

Chemical disinfection of water is a common procedure aiming to control waterborne infectious diseases. Nevertheless, different reactions occur between the organic matter present in raw water and the chemicals used to disinfect it (Banach et al., 2015). As result of these reactions, disinfection by-products (DBPs) are produced. DBPs constitute a complex mixture of many different chemical groups, with a number of compounds presenting mutagenic and carcinogenic risk (Richardson et al., 2007). Among them, we can point out haloacetic acids (HAAs) (Xue et al., 2016), which have become a public health concern due to their potential toxic and carcinogenic risk (Pals et al., 2011). Among HAAs, chloroacetic acids (CAAs), bromoacetic acids (BAAs), and iodoacetic acids (IAAs) are found with an important prevalence in disinfected water (Krasner et al., 2006).

Due to their potential impact on public health, some DBPs are regulated in many countries. HAAs are the second most abundant species amidst halogenated DBPs, ranging from mid- to sub-micromolar concentrations (Richardson et al., 2007). Among this DBPs class, only five (bromoacetic acid, dibromoacetic acid, chloroacetic acid, dichloroacetic acid, and trichloroacetic acid) are currently regulated by the U.S. EPA, with a maximum contaminant level (MCL) for the sum of the five regulated HAAs of 60 μg/L (Environmental Protency Agency, 2006). One of the long-term human health implications produced by DBPs exposure is cancer (Grellier et al., 2015), with bladder cancer being one of the main documented effects in epidemiological studies (Villanueva et al., 2015; Hrudey et al., 2015). Nevertheless, the potential carcinogenic risk of each individual DBP remains to be determined.

Long-term studies of carcinogenesis using mammalian models are lengthy and expensive, and present important ethical implications. For this reason the *in vitro/in vivo* genotoxic assessment of DBPs, by using different biomarkers as chromosome damage, micronuclei, or telomeric instability, are usually accepted as a surrogate biomarker of their potential cancer risk (Richardson et al., 2007; Liviac et al., 2011; Manasfi et al., 2015; Teixidó et al., 2015). Nevertheless, this approach underestimates the risk of non-genotoxic carcinogens, implying that a more direct way to measure the potential carcinogenic risk of individual DBPs *in vitro* remains to be developed.

To overcome this problem, *in vitro* cell transformation assays (CTA)

* Corresponding author at: Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Campus de Bellaterra, 08193, Cerdanyola del Vallès, Barcelona, Spain.
E-mail address: ricard.marcos@uab.es (R. Marcos).

https://doi.org/10.1016/j.taap.2018.04.004
Received 17 October 2017; Received in revised form 30 March 2018; Accepted 3 April 2018
Available online 07 April 2018
0041-008X/ © 2018 Published by Elsevier Inc.

have been proposed as suitable alternatives to long-term animal studies to measure carcinogenic effects. Cellular and molecular processes involved in *in vitro* cell transformation are close to those taking place in *in vivo* carcinogenesis (Creton et al., 2012). It is accepted that an exposed cell line becomes tumorigenic when different cancer hallmarks become evident (Hanahan and Weinberg, 2011). Among the different hallmarks of cell transformation, morphological cell changes, anchorage-independent cell growth, secretion of matrix metalloproteinases (MMPs), and invasiveness have been used in different studies (Tokar et al., 2013; Garrett et al., 2014; Laulicht et al., 2015; Annangi et al., 2016). The Organization for Economic Co-operation and Development (OECD) analyzed the performance of three CTAs to screen the carcinogenic potential of chemicals. The Syrian hamster embryo (SHE) cells, and the mouse cell lines BALB/c 3T3 and C3H10T1/2 were evaluated and, as a conclusion, the development of an OECD guideline with the SHE and BALB/c 3T3 models was proposed (Vasseur and Lasne, 2012). However, the acute treatment methodology employed by these assays may not reproduce the actual case scenario, where target cells are exposed to low doses of carcinogenic agents for a long period of time. This could be translated as an overestimation of the actual carcinogenic potential of chemical species which do not pose health risks at realistic concentrations. Due to the aforementioned reasons, the aim of this study has been to determine the potential carcinogenic hazard of three HAAs, namely chloroacetic acid (CAA), bromoacetic acid (BAA) and iodoacetic acid (IAA) under an experimental approach that tries to mimic a realistic exposure scenario. To this aim, long-term exposures (8 weeks) and low, non-cytotoxic concentrations in a range found in disinfected water, were used (Ding et al., 1999; Loos and Barceló, 2001; Richardson et al., 2008). The human bladder cell line T24 was used as study model since bladder cancer is one of the main effects associated with DBPs exposure. Previous studies in our lab have pointed out the low transforming potential of other families of DBPs over non-transformed cell lines (Marsà et al., 2017). The use of a transformed cell line such as T24 should overcome this issue, potentiating the already expressed tumorigenic markers in case of a carcinogenic input (Sun et al., 2017).

From the three selected HAAs, chloroacetic acid was reported as non-carcinogenic in mouse and rat (NTP, 1992); nevertheless, dichloro- and trichloroacetic acids were classified as carcinogenic, promoting liver tumors in mice (Tao et al., 1998). Since the number of chlorine atoms changes the carcinogenic potential of these HAAs, it is plausible that changing chlorine for bromine or iodine atoms can modify the carcinogenic potential; especially taking into account that the presence of iodine or bromine atoms increase the toxicity and genotoxicity of HAAs (Plewa et al., 2010).

2. Materials and methods

2.1. Cell culture conditions and in vitro DBP exposure

The human bladder carcinoma cell line T24 and the human cervix epitheloid carcinoma cells (HeLa) were maintained in DMEM high glucose medium (LifeTechnologies, NY, USA) supplemented with 10% fetal bovine serum (FBS; PAA®, Pasching, Austria), 1% of non-essential amino acids (NEAA; PAA®) and 2.5 μg/mL plasmocin (InvivoGen, CA, USA) at 37 °C in a humidified 5% CO_2 incubator. Chloroacetic acid (CAA, $C_2H_3ClO_2$, CAS 79–11-8) and bromoacetic acid (BAA, $C_2H_3BrO_2$, CAS 79-08-3) were both purchased from Acros Organics (Geel, Belgium). Iodoacetic acid (IAA, $C_2H_3IO_2$, CAS 64-69-7) was purchased from Sigma-Aldrich (MO, USA). Three separate 75-cm^2 flasks of T24 cells were exposed to non-cytotoxic concentrations of the three HAAs (10 and 100 μM CAA, 0.005 and 0.05 μM BAA; 0.01 and 0.1 μM IAA) for 8 weeks, the DBP-containing medium was changed every 72 h, and subconfluent cells were passaged weekly. CAA was diluted in distilled water to an initial concentration of 100 mM. BAA initial concentration was 50 mM, and for IAA was 10 mM. In all cases, working concentrations were calculated, and freshly prepared before every treatment; but were not directly measured after HAA addition to the media. The manipulation of chemicals was conducted in a certified biological/chemical safety hood following the manufacturer's instructions.

2.2. Analysis of cell viability

T24 cells were plated in opaque 96-well plates in triplicates at a density of 20,000 cells per well and incubated overnight in complete medium. Next, it was replaced with fresh medium with concentrations up to 10 mM of BAA, CAA or IAA. Twenty-four h after the exposure, cells were washed with PBS and incubated in 0.44 μM of resazurin dye (Alfa Aesar, Karlsruhe, Germany) for 2 h. Fluorescence emitted by the reduction product resorufin was then measured using a microplate fluorimeter equipped with a 560 nm excitation/590 nm emission filter set, being the resorufin produced proportional to the number of viable cells. Cytotoxicity curves derived from averaging three independent experiments and the IC_{50} values were calculated using GraphPad prism version 7.00. The same protocol was followed to determine whether the 8 weeks treatment induced variations in cells resistance to an agent inducing oxidative damage. T24 cells previously exposed to the higher concentrations of the three HAAs for 8 weeks were exposed to increasing concentrations of $KBrO_3$ (3 mM to 15 mM) for 24 h. Resazurin incubation and following data analysis were performed as described previously.

2.3. Determination of genotoxic and oxidative DNA damage

Genotoxic and oxidative DNA damage were assessed by the alkaline comet assay, with and without the use of formamidopyrimidine DNA glycosylase (FPG) enzyme, in T24 cells exposed to the HAAs for 4 h and 8 weeks. The comet assay detects single- and double-stranded DNA breaks in naked supercoiled DNA. These DNA strand breaks allow loops of DNA to migrate during the electrophoresis, forming a comet tail. The use of enzymes as FPG allows the detection of oxidative damage. Untreated and long-term exposed T24 cells were seeded in 6-well plates in triplicates at a density of 200,000 cells per well. Untreated cells were then exposed to concentrations ranging from 750 to 1500 μM for CAA, 5 to 25 μM for BAA, and 2.5 to 8 μM for IAA; while long-term exposed cells remained exposed to the long-term treatment. To assess a possible resistance to oxidative damage after the long treatments, long-term exposed cells were as well exposed to 2.5 mM $KBrO_3$ for 30 min. After the corresponding exposure times, cells corresponding to positive controls were incubated 30 min in 200 μM MMS and 5 mM $KBrO_3$. At the end of the treatment, cells were washed twice with PBS and collected by trypsinization. A mixture 1:10 containing the cells and 0.75% agarose at 37 °C was then prepared, and 7 μL drops were placed onto a Gelbond® film (GBF, 10.5 × 7.5 cm), with 3 replicate drops corresponding to each treatment. Two identical GBF were processed simultaneously for each experiment. Both films were then lysed overnight by immersion in ice-cold lysis buffer (2.5 M NaCl, 0.1 M Na_2EDTA, 0.1 M Tris base, 1% Triton X-100, 1% lauroyl sarcosinate, 10% DMSO, pH 10) at 4 °C. One film was incubated for 30 min at 37 °C in enzyme buffer containing FPG and the other one in enzyme buffer alone. Both films were washed with electrophoresis buffer (0.3 M NaOH and 1 mM Na_2EDTA, pH 13.2), and placed into a horizontal gel electrophoresis tank. Films were incubated for 35 min in the same buffer to allow DNA unwinding prior to electrophoresis, performed at 1 V/cm and 300 mA for 20 min at 4 °C. After the electrophoresis, both films were rinsed with cold PBS for 15 min, fixed in absolute ethanol for 2 h and air-dried overnight at room temperature. Prior to observation, GBF were stained for 20 min with 1/10,000 diluted SYBR Gold (Molecular Probes). Once mounted on an acrylic slide and covered with an appropriate coverslip, comet tails were measured using the Komet 5.5 Image analysis system (Kinetic Imaging Ltd., Liverpool, UK). 100 cells were scored for each treatment, and the percentage of DNA in tail was the parameter used to measure the DNA damage.

Download English Version:

https://daneshyari.com/en/article/8538615

Download Persian Version:

https://daneshyari.com/article/8538615

Daneshyari.com