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Monohaloacetic acid drinking water disinfection by-products inhibit follicle growth and steroidogenesis in mouse ovarian antral follicles *in vitro*

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ABSTRACT

Water disinfection greatly reduced the incidence of waterborne diseases, but the reaction between disinfectants and natural organic matter in water leads to the formation of drinking water disinfection by-products (DBPs). DBPs have been shown to be toxic, but their effects on the ovary are not well defined. This study tested the hypothesis that monohalogenated DBPs (chloroacetic acid, CAA; bromoacetic acid, BAA; iodoacetic acid, IAA) inhibit antral follicle growth and steroidogenesis in mouse ovarian follicles. Antral follicles were isolated and cultured with either vehicle or DBPs (0.25–1.00 mM of CAA; 2–15 μM of BAA or IAA) for 48 and 96 h. Follicle growth was measured every 24 h and the media were analyzed for estradiol levels at 96 h. Exposure to DBPs significantly inhibited antral follicle growth and reduced estradiol levels compared to controls. These data demonstrate that DBP exposure caused ovarian toxicity *in vitro*.

1. Introduction

The treatment of water using disinfectants such as chlorine, ozone, chloramines, chlorine dioxide, and ultraviolet radiation greatly reduced the incidence of waterborne diseases, including cholera and typhoid and is considered a major public health achievement of the 20th century [1]. However, the reaction between disinfectants and natural organic as well as inorganic matter in the source water can lead to an unintended consequence, which is the formation of drinking water disinfection by-products (DBPs) [2,3]. Trihalomethanes were the first DBP chemical class discovered in 1974 [4,5]. To date, more than 600 DBPs have been identified in finished drinking waters [3]. In 2006, the U.S. EPA issued the Stage 2 Disinfectants (D)/DBP Rule to control the maximum contaminant levels of 11 DBPs, including four trihalomethanes, five haloacetic acids (HAAs), bromate (BrO_3^-), and chlorite (ClO_2^-) [6]. The five regulated HAAs include chloroacetic acid (CAA), dichloroacetic acid, trichloroacetic acid, bromoacetic acid (BAA), and dibromoacetic acid at a maximum contaminant level of 60 μg/L for their sum [6]. Unregulated HAAs include iodoacetic acid (IAA), bromochloroacetic acid, tribromoacetic acid, bromodichloroacetic acid, and chlorodibromoacetic acid [7].

Many DBPs are cytotoxic, genotoxic, mutagenic, and teratogenic [8]. Epidemiological studies demonstrate an association between lifetime exposures to DBPs and increased risk of cancers [9–12]. Further, some studies show an association between DBP exposure and adverse pregnancy outcomes such as low birth weight, small-for-gestational age, still birth, and birth defects [13–20]. Because the HAAs are the most regulated chemical class of DBPs, many studies focused on their toxicological impacts both *in vitro* and *in vivo*. The monohaloacetic acids (monoHAAs) modulate gene expression involved in the stress response to DNA damage, cell cycle regulation, the induction of reactive oxygen species, and apoptosis in non-transformed human intestinal cells [21–23]. IAA exposure induces malignant transformation of NIH/3T3 xenografts in Balb/c nude mice that progress to highly aggressive fibrosarcomas [24]. MonoHAAs affect CD-1 mouse embryos and induce

Abbreviations: BAA, bromoacetic acid; CAA, chloroacetic acid; CHO, Chinese hamster ovary; DBP, disinfection by-product; DMSO, dimethylsulfoxide; E2, estradiol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IAA, iodoactic acid; α-MEM, α-minimal essential medium; ROS, reactive oxygen species.

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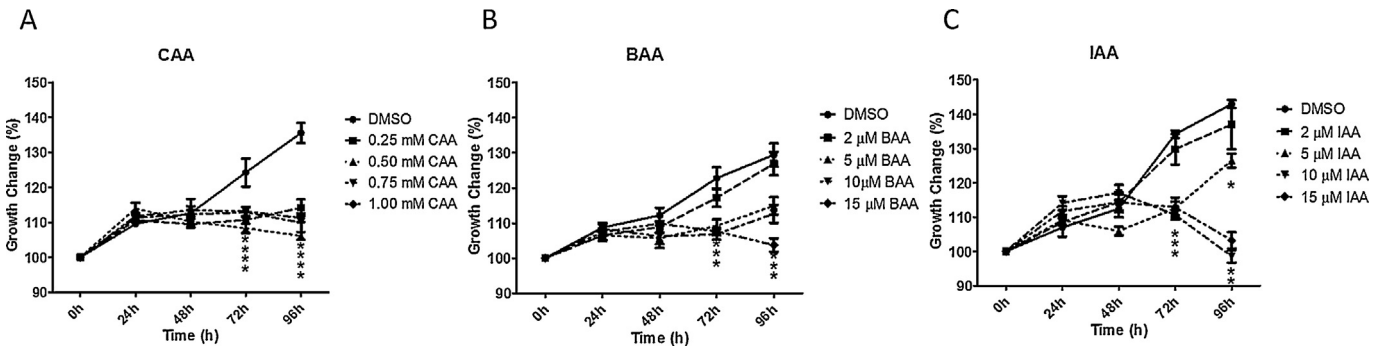

Fig. 1. Effect of CAA, BAA or IAA treatment on antral follicle growth.
Mechanically isolated antral follicles were cultured with CAA, BAA, or IAA for 96 h. Growth of follicles was recorded in micrometers every 24 h and reported as percent change compared to the follicle size at the beginning of treatment (0 h = 100%). DMSO = dimethylsulfoxide, CAA = chloroacetic acid, BAA = bromoacetic acid, IAA = iodoacetic acid. Data represent means ± SEM from at least three separate experiments. Asterisks (*) represent significant differences from DMSO control at each time point ($p \leq 0.05$).

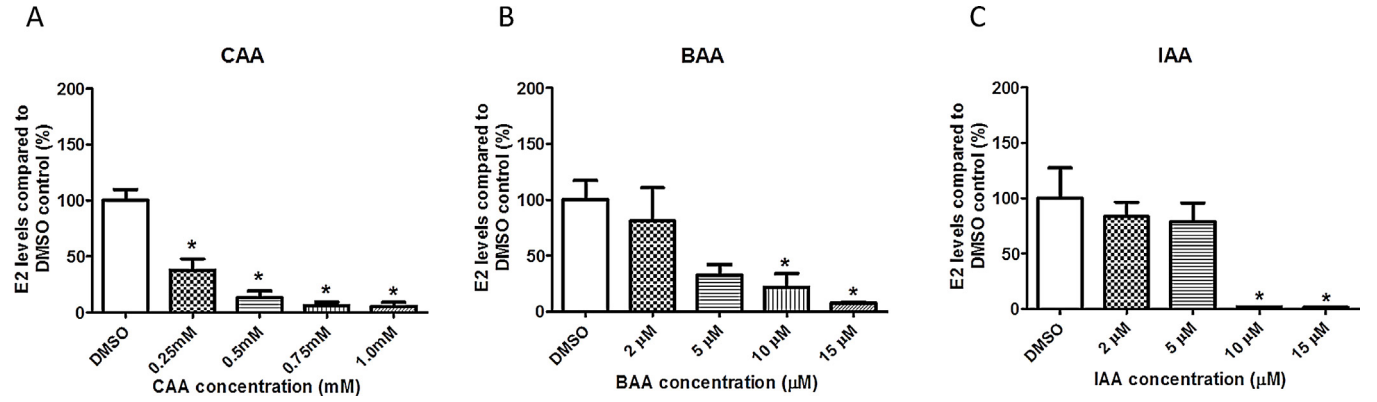

Fig. 2. Effect of CAA, BAA or IAA treatment on estradiol levels.
Antral follicles were cultured with CAA, BAA or IAA for 96 h. After culture, media were collected and analyzed for estradiol levels. DMSO = dimethylsulfoxide, CAA = chloroacetic acid, BAA = bromoacetic acid, IAA = iodoacetic acid. Data represent means ± SEM from at least three separate experiments. Asterisks (*) represent significant differences from DMSO control ($p \leq 0.05$).

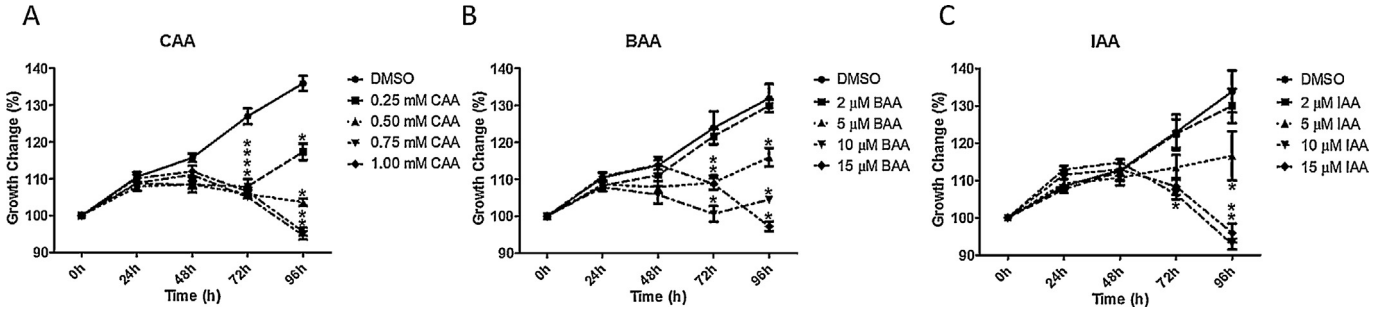

Fig. 3. Effect of acute 48 h-exposure of CAA, BAA or IAA on follicle growth .
Mechanically isolated antral follicles were cultured with CAA, BAA or IAA. Chemicals were removed after 48 h and follicles were cultured for an additional 48 h with fresh supplemented α-MEM (total 96 h of culture). Growth of follicles was recorded in micrometers every 24 h and reported as percent change compared to the follicle size at the beginning of culture. DMSO = dimethylsulfoxide, CAA = chloroacetic acid, BAA = bromoacetic acid, IAA = iodoacetic acid. Data represent means ± SEM from at least three separate experiments. Asterisks (*) represent significant differences from DMSO control at each time-point ($p \leq 0.05$).

dysmorphogenesis in neural tube and eye development and produce anomalies in heart development [25]. Several dihaloacetic acids alter intestinal microbial populations and their metabolism, which could lead to bioactivation of promutagens or procarcinogens in rats [26]. Dibromoacetic acid alters spermatogenesis and disrupts testicular steroidogenesis in male rats [27,28]. In addition, dibromoacetic acid disrupts estrous cyclicity and suppresses estradiol catabolism, which leads to alterations in steroid production in female rats [29,30].

Although previous studies indicate that DBPs are toxicants in many systems, the effects of the monoHAAs on the ovary are largely unknown. The ovarian follicle is the functional unit of the ovary that is responsible for growth of the oocyte and the production of sex steroid hormones. Antral follicles are the most mature type of mammalian ovarian follicles and are the major source of sex steroid hormone production. Because antral follicles are the only follicle type capable of ovulation and the major producers of estradiol (E2), alterations in proper growth or function of the antral follicle may result in reduced fertility or abnormal steroidogenesis. Therefore, this study tested the hypothesis that the monoHAAs (CAA, BAA, and IAA) directly affect follicle growth and E2 production in mouse antral follicles *in vitro*.