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Joint effects of trihalomethanes and trichloroacetic acid on semen quality: A population-based cross-sectional study in China☆

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ABSTRACT

Exposure to trihalomethanes (THMs) and haloacetic acids (HAAs) has been individually associated with adverse male reproductive effects; however, their joint male reproductive toxicity is largely unknown. This study aimed to explore the joint effects of THMs and trichloroacetic acid (TCAA) on semen quality in a Chinese population. A total of 337 men presenting to the Reproductive Center of Tongjing Hospital, in Wuhan, China to seek semen analysis were included this study. Baseline blood THMs [chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform (TBM)] and urinary TCAA were analyzed and dichotomized at their median levels. The joint effects of THMs and TCAA on below-reference semen quality parameters were evaluated by calculating the relative excess risk due to interaction (RERI). After adjusting for potential confounders, we found a suggestive synergistic effect between Br-THMs (sum of BDCM, DBCM, and TBM) and TCAA for below-reference sperm count (RERI = 2.14, 95% CI: −0.37, 4.91) (P = 0.076); men with high Br-THMs and TCAA levels (above the median) had 3.31 times (95% CI: 1.21, 9.07) elevated risk of having below-reference sperm count than men with low Br-THMs and TCAA levels (below the median). No apparent joint effects were observed between THMs and TCAA for other semen quality parameters. Our results suggest that co-exposure to Br-THMs and TCAA is associated with additive effects on decreased semen quality. However, further studies in a larger sample size and mechanistic studies are needed to confirm the findings.

1. Introduction

Disinfection of drinking water has been proven to play a determinate role in reducing the incidence of waterborne diseases. However, harmful disinfection by-products (DBPs) are inevitably formed when disinfectants react with naturally occurring organic matters and inorganic chemicals in the raw water. To date, more than 600 kinds of DBPs have been identified in drinking water, of which the two most prevalent classes are trihalomethanes (THMs) and haloacetic acids (HAAs) (Ding et al., 2013; Richardson et al., 2007). Humans can be exposed to DBPs through ingestion, inhalation, and dermal absorption during routine water-use activities such as drinking, bathing, showering, and swimming (Nieuwenhuijsen et al., 2009). Potential adverse health effects of exposure to drinking water DBPs have been a public health concern (Grellier et al., 2015).

Animal studies have demonstrated that exposure to THMs or HAAs can cause adverse male reproductive effects, including reduced reproductive organ weights, impaired reproductive competence, and decreased semen quality (Klinefelter et al., 1995; Linder et al., 1995, 1997; Veeramachaneni et al., 2007). Epidemiological studies utilized DBP concentrations in drinking water as surrogates of exposure and reported varying effects of exposure to DBPs on semen quality (Fenster et al., 2003; Iszatt et al., 2013; Luben et al., 2007; Zeng et al., 2014a). A lack of accurate exposure assessment in these studies may attenuate the exposure-related outcomes (Arbuckle et al., 2002). Our recent studies used the

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biomarkers of DBPs to improve exposure assessment and found that baseline blood THMs were associated with decreased sperm concentration and count (Zeng et al., 2013), and that urinary trichloroacetic acid (TCAA) was associated with decreased sperm concentration, motility, and count (Zeng et al., 2014b).

Although exposure to THMs or HAAs has been individually associated with adverse male reproductive effects, limited studies evaluate the combined reproductive toxicity of any mixtures of these two compounds. In reality, DBPs exist as complex mixtures in drinking water. Humans are concurrently exposed to mixtures of drinking water DBPs through various routes. A previous animal study in adult rats reported that exposure to two binary mixtures of dibromoacetic acid (DBAA) and bromochloroacetic acid (BCAA) synergistically decreased the levels of SP22, a sperm membrane protein that is highly correlated with male fertility (Kaydos et al., 2004). In a recent multigenerational study, Narotsky et al. (2015) observed that exposure to a mixture of THMs [chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform (TBM)] and HAAs (chloroacetic acid, dichloroacetic acid, TCAA, bromoacetic acid, and DBAA) had an increased incidence of compromised sperm motility in F1 adult male rats. However, the human evidence on the joint male reproductive toxicity of any mixtures of THMs and HAAs has not been reported to date.

In the present study, we took advantage of the data from a cross-sectional study of exposure to drinking water DBPs and male reproductive health in China, in which two biomarkers of DBPs, including THMs in whole blood and TCAA in urine, were analyzed. The blood biomarker can represent an integrative measure of THM exposure from various routes (Miles et al., 2002). The urine biomarker has been proposed as a valid biomarker of TCAA ingestion through chlorinated drinking water (Kim et al., 1999; Zhang et al., 2009a). The primary aim of this study was to explore the joint effects of THMs and TCAA on semen quality.

2. Materials and methods

2.1. Study subjects

The study subjects included in this study were from men who participated in an ongoing cross-sectional study of exposure to drinking water DBPs and male reproductive health in Wuhan, China (Zeng et al., 2014b). Briefly, a total of 2540 men presenting to the Reproductive Center of Tongjing Hospital to seek semen analysis agreed to participate in the study between April 2011 and May 2012, of whom 356 men provided urine and blood samples. We excluded 14 azoospermic men because the mechanism responsible for azoospermia may be related to an obstructive mechanism or Y-chromosome deletions. We also excluded 5 men who reported occupational exposure to chemicals (e.g., trichloroethylene, 1,1,1-trichloroethane, and perchloroethylene) because these chemicals can be metabolized into TCAA. After these exclusions, a total of 337 men were available for the current analysis. All the study subjects completed a face-to-face questionnaire at enrollment, and the collection of information included demographics, lifestyle habits, occupational exposures, medical characteristics, and routine water-use activities. The study was approved by the Ethics Committee of Tongji Medical College, and each study subject signed an informed consent after receiving an explanation about the study procedures.

2.2. Blood THM analysis

We collected 5-mL peripheral blood in the morning using a gray-cap tube that was specially treated to eliminate background THM contamination (Cardinali et al., 1995). Morning blood samples collected prior to major water-use activity (e.g., bathing, showering, and swimming) can be expected to yield baseline THM concentrations (Rivera-Nunez et al., 2012). The analytical method used to measure baseline blood THM concentrations has been described in detail in our previous study (Zeng et al., 2013). Briefly, volatiles were extracted from 3-mL blood sample headspace onto a solid phase micro-extraction (SPME) fiber and then desorbed in the gas chromatography (GC) inlet followed by detection using an electron capture detector (ECD). Each analysis run (20–30 samples) included two blanks (a laboratory air sample and a blank water sample) and two quality control samples (serum spiked with THMs). The limit of detections (LOD) for TCM was 1.95 ng/L, 0.45 ng/L for BDCM, 0.68 ng/L for DBCM, and 2.00 ng/L for TBM.

2.3. Urine TCAA analysis

We collected a spot urine sample in 50-mL conical polyethylene container from each study subject in the morning. Urine TCAA concentrations were analyzed according to the method that has been described in detail elsewhere (Xie et al., 2011). Briefly, a 10-mL urine sample was extracted using methyl-tert-butyl-ether. After centrifugation at 4 °C for 5 min, TCAA in organic extraction was converted to its methyl ester by the addition of acidic methanol followed by heating at 50 °C for 2 h. Then, the target analyte was analyzed using the GC coupled with an ECD. One blank and two quality control samples (urine spiked with TCAA) were also analyzed along with each analysis run (30–40 samples). The LOD for urinary TCAA was 2.00 μg/L. Urinary creatinine was determined to adjust for the variation in urine diluteness by the picric acid assay using commercial test kits.

2.4. Semen analysis

The study subjects were asked to masturbate into a sterile plastic specimen container in a specialized semen collection room. Semen samples were liquefied in a heating chamber (37 °C) for no more than 60 min before analysis. Semen quality parameters were analyzed according to the World Health Organization (WHO, 1999) guidelines that have been described in detail elsewhere (Zeng et al., 2015). Briefly, semen volume was measured using a serologic pipette. Sperm concentration (million/mL) and motility (% A + B motile sperm) were analyzed using a Micro-cell slide and computer-aided semen analysis. Sperm count (million) was calculated by multiplying the semen volume by the sperm concentration. Quality controls were established based on the WHO guidelines and supervised by the Quality Control Center of Hubei Province.

2.5. Statistical analysis

The concentrations of target analytes below the LODs were replaced with the LOD divided by the square root of 2. The brominated THMs (Br-THMs) were obtained by summing BDCM, DBCM, and TBM in blood. The total THMs (TTHMs) were obtained by summing TCM, BDCM, DBCM, and TBM in blood. We calculated the descriptive characteristics on the distributions of characteristics by semen parameters, as well as baseline blood THM and urinary TCAA concentrations. Semen parameters were dichotomized as either below or at/above the WHO reference values (WHO, 1999) for sperm concentration (20 million/mL), sperm motility (50% motile), and sperm count (40 million). Subjects with all three parameters at or above the reference values were defined as the comparison group. We used Spearman correlation coefficients to examine the correlations among blood THMs and creatinine-adjusted urinary TCAA concentrations.