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Evaluation of genotoxic effects caused by extracts of chlorinated drinking water using a combination of three different bioassays

Qiang Zeng[a,b], Shao-Hui Zhang[a,c], Jing Liao[a,b], Dong-Yue Miao[a,b], Xin-Yi Wang[a,b], Pan Yang[a,b], Luo-Jia Yun[a,b], Ai-Lin Liu[a,b,1], Wen-Qing Lu[a,b,*,1]

[a] Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China
[b] Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China
[c] Experiment Center, The Third Hospital of Hebei Medical University, Shijiazhuang, Hebei, PR China

HIGHLIGHTS

- Genotoxic effects of water extracts were evaluated using a combination of three different bioassays.
- All water samples in January and July induced at least one types of genotoxic effects.
- The levels of gene-mutation and DNA-damage effects in January were higher than those in July.
- Chlorination increased the different types of genotoxic effects of drinking water that was dependent on the sampling times.

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ABSTRACT

Potential genotoxic effects of chlorinated drinking water now are of a great concern. In this study, raw water, finished water, and tap water from a water plant in Wuhan, China were collected in two different sampling times of the year (January and July). Genotoxic effects of water extracts were evaluated using a combination of three different bioassays: SOS/umu test, HGPRT gene mutation assay, and micronucleus assay, which were separately used to detect DNA damage, gene mutation, and chromosome aberration. The results of three different bioassays showed that all water samples in January and July induced at least one types of genotoxic effects, of which the DNA-damage effects were all detectable. The levels of DNA-damage effects and gene-mutation effects of finished water and tap water in January were higher than those in July. Chlorination could increase the DNA-damage effects of drinking water in January and the gene-mutation effects of drinking water in both January and July, but did not increase the chromosome-aberration effects of drinking water in both January and July. Our results highlighted the importance of using a combination of different bioassays to evaluate the genotoxicity of water samples in different seasons.

1. Introduction

A number of epidemiological studies have suggested that consumption of chlorinated drinking water is associated with increased cancer risks and adverse reproductive outcomes [1–5]. These findings have led to an increasing concern on potential genotoxic effects of chlorinated drinking water in water hygiene and public health. It has been reported that genotoxic chemicals present in chlorinated drinking water originate from two major sources: (1) raw water used as source for water supply is ubiquitously polluted by a variety of genotoxic contaminants due to their direct or indirect discharges after industrial, domestic, and agricultural usages [6]; (2) numerous mutagenic and/or carcinogenic disinfection by-products (DBPs) are formed during the chlorination of drinking water as a result of the reaction between disinfectants and naturally occurring organic matters and anthropogenic contaminants in water [7].

* Corresponding author at: Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China. Tel.: +86 27 83610149; fax: +86 27 83657765.
E-mail address: luwq@mails.tjmu.edu.cn (W.-Q. Lu).
[1] These two authors equally contributed to this work.

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A larger number of studies have reported the genotoxic effects of raw water and chlorinated drinking water in different countries [8–11]. Because genotoxic chemicals in the water occur temporally, seasonal variations on genotoxic effects of raw water and chlorinated drinking water have also been reported in numerous studies [12–15]. However, due to different classes of genotoxic compounds and/or complex chemical constituents, water samples generally induce multiple types of genetic endpoints, including DNA damage, gene mutation, and chromosome aberration [11,16,17]. Therefore, a combination of different bioassays to detect multiple genetic endpoints of water samples may provide a comprehensive assessment [18].

Hanjiang River, as the largest tributary of the Yangtze River in central China, is one of the main drinking water sources for local water plants in Wuhan city. Conventional chlorination method is applied in all water plants of Wuhan city. Recently, as the rapid development in economy and increase in population, a multitude of industrial and household wastewater are directly or indirectly released into Hanjiang River, resulting in a serious pollution and eutrophication of drinking water source [17,19,20]. In our previous study using the comet assay, the genotoxic effect caused by extract of chlorinated drinking water from Hanjiang River was more serious than that from Yangtze River [21]. However, a comprehensive of assessment on the genotoxic effect of chlorinated drinking water from Hanjiang River has been rarely reported.

In the present study, a combination of three different bioassays, including SOS/umu test, HGPRT gene mutation assay, and micronucleus assay, that detect different types of genetic endpoints was used to comprehensively evaluate the genotoxic effects of raw water, finished water, and tap water extracts from a water plant in Wuhan city, China. The SOS/umu test is less time-consuming and labor-intensive compared with the comet assay, which has been extensively and effectively used to detect the DNA-damage effects of various genotoxic substances and complex water samples [14,22,23]. The HGPRT gene mutation assay is an excellent microplate-based assay to detect the gene-mutation effects of various compounds including DBPs [24,25]. The micronucleus assay, a multi-end-point assay, detects chromosome breakage, chromosome loss, and chromosome rearrangement, which has been widely to evaluate the chromosome-aberration effects of complex water samples [17,26].

2. Materials and methods

2.1. Water sample collection and preparation

Fifty liters of raw water, finished water, and tap water from a water plant in Wuhan city were collected in January (winter) and July (summer) 2009, which represent the strong variation in water quality throughout China. The water quality of Hanjiang River is generally deteriorated in winter [12,13]. The conventional chlorination process (pre-chlorination → coagulation → flocculation → filtration → post-disinfection) is applied in the water plant. The raw water sample was from Hanjiang River. The finished water sample was after post-disinfection but before water distribution system. The tap water sample was from the water distribution system. After collection, all the water samples were immediately transported to the laboratory and acidified with HCl (pH 2). The water-quality parameters including pH, water temperature, and free residual chlorine were measured for each water sample according to China Water Quality Standards for Urban Water Supply [27].

Water extracts were prepared using solid-phase extraction that has been described in detail in our previous study [28]. In brief, water samples were extracted using Amberlite XAD-2 resins that were pre-cleaned with methanol, hexane, acetone, and dichloromethane. After water sample filtration at a rate of 30 mL/min, the resins were eluted with hexane and acetone mixture. Finally, the water extracts were evaporated to dryness using a rotary evaporator and then dissolved in dimethyl sulfoxide (DMSO) to concentration corresponding to 50 L water per mL DMSO. The all water extracts were stored at −20 °C until testing.

2.2. SOS/umu test

The strain (*Salmonella typhimurium* TA1535/pSK1002) was provided by Tokyo University of Pharmacy and Life Sciences (Japan). The SOS/umu test without S9 metabolic activation was performed according to a previous method [29] with minor modifications. The strain was cultured overnight in 10-mL TGA medium at 37 °C with shaking. The next day, the overnight culture medium was diluted 10-fold with fresh TGA medium and incubated at 37 °C with shaking for 1.5 h. Then, mixture in 96 well plates including 20-μL10-fold TGA medium, 70-μL strain culture, and 180-μLwater extracts (final doses ranged from 30 to 2000 mL water/mL medium) was incubated at 37 °C with shaking for 2 h. The TGA medium and DMSO were used as blank control and negative control, respectively. Each dose of water extracts was performed in triplicates. After incubation, 30-μL mixture was diluted with 270-μL TGA medium, followed by a re-incubation for 2 h at 37 °C with shaking, and the absorbance value at 600 nm was measured. After measurement, 120-μL Z-buffer, 30-μL *o*-nitrophenyl-β-D-galactopyranoside, and 30-μL re-incubation mixture were mixed to initiate the enzyme reaction at 30 °C with shaking for 0.5 h. The enzyme reaction was terminated by adding 120-μL Na_2CO_3 (1 M), and the absorbance value at 420 nm was measured. The genotoxic potential was expressed as the β-galactosidase activity. The growth ratio (GR) and induction ratio (IR) were calculated according to the following equation:

$$\mathrm{GR} = \frac{A_{600,\mathrm{T}} - A_{600,\mathrm{B}}}{A_{600,\mathrm{N}} - \mathrm{A}_{600,\mathrm{B}}}$$

$$\mathrm{IR} = \frac{A_{420,\mathrm{T}} - A_{420,\mathrm{B}}}{A_{420,\mathrm{N}} - A_{420,\mathrm{B}} \times \mathrm{GR}}$$

where GR less than 0.5 indicated the cytotoxicity of water extract on *S. typhimurium* TA1535/pSK1002 [23]; IR greater than 2.0 indicated the DNA-damage effects in test water extracts [14]; $A_{600,\mathrm{T}}$, $A_{600,\mathrm{B}}$, and $A_{600,\mathrm{N}}$ were the absorbance values of test water extracts, blank control, and negative control at 600 nm, respectively; $A_{420,\mathrm{T}}$, $A_{420,\mathrm{B}}$, and $A_{420,\mathrm{N}}$ were the absorbance values of test water extracts, blank control, and negative control at 420 nm, respectively.

Genotoxicity potency (GP), the concentration of water extract that induced an IR of 2.0, was used to quantitatively compare the DNA-damage effects of water extracts (the lower GP value indicated the stronger DNA-damage effect). The GP was calculated using the Tablecurve 2D software (version 5.01, Systat Software Inc.).

2.3. HGPRT gene mutant assay

The Chinese hamster ovary K1 (CHO-K1) cell was provided by Shanghai Institute of Cell Biology, Chinese Academy of Sciences. The cells were grown in Ham's F12 medium containing 10% fetal bovine serum (FBS), 100-U penicillin/mL, and 100-μg streptomycin/mL at 37 °C and 5% CO_2. The HGPRT gene mutation assay without exogenous metabolic activation was performed according to the method as described in our previous study [25]. Briefly, a density of 1×10^6 CHO-K1 cells was inoculated in a dish containing Ham's F12 medium (10% FBS). After 24 h incubation at 37 °C, the medium was replaced with serum- and antibiotics-free medium