Genotoxicity of drinking water from three Korean cities


Abstract

Organic content of drinking tap water from Seoul, Taejon, and Suwon was extracted with an XAD-2 resin column and organic solvents. Four doses of the extract equivalent to 4, 2, 1, and 0.5 l water were tested for mutagenicity in Salmonella typhimurium strains TA98 and TA100 in the presence and absence of S9 mix. The organic extracts of the water from all three cities were mutagenic in TA98 without S9 mix and in TA100 with and without S9 mix. The highest number of revertants per plate was found in the absence of S9 mix. Three doses of the extract (equivalent to 22, 11, and 3.7 l water) were also tested in the bone marrow micronucleus test using BDF1 mice. At the highest dose, a significant increase of the micronucleus frequency was observed. The time required to be on the effect, however, varied with the source of the water. Our results indicate that the drinking tap waters from the three cities were genotoxic clearly in the bacterial test and also in the in vivo assay with mice. As we found no genotoxicity of the source water as seen in a previous study, it is likely that the chlorination process leads to the genotoxicity of the tap water. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Drinking water; Mutagenicity; Genotoxicity; Micronuclei; The Ames test

1. Introduction

Rivers and lakes are the major sources of water used for drinking water as well as for recreational and agricultural purposes in Korea. The potential genotoxicity of such water due to contamination with industrial and domestic waste, agricultural products, and potentially reactive natural products is an area of active study [1–4]. In addition, chlorination of drinking water as a disinfection process can generate halogenated by-products such as trihalomethanes (THMs) and nonvolatile mutagens, which are associated with health risks, including cancers [5–8].

The mutagenic activity of chlorinated drinking water has been shown in numerous studies [8,9]. The Ames (Salmonella/microsome) test is widely used as a screening test for the evaluation of chemicals for mutagenicity [10,11]. Meanwhile, the mouse bone marrow micronucleus test is an in vivo assay for the evaluation of chemicals for genotoxicity including
chromosomal damage and chromosome loss [12,13]. Micronuclei originate from acentric chromosome fragments as well as from whole chromosomes lagging at anaphase.

In Korea, most people in the cities drink only purified or filtered water because they assume that the water from municipal water-treatment plants is contaminated with industrial and agricultural waste products and is not safe. In the present study, we evaluated if the assumption is correct by assaying the genotoxicity of drinking water samples from three major cities using the Salmonella/microsome test and the mouse bone marrow micronucleus test. This report describes our findings.

2. Materials and methods

2.1. Sample preparation

Drinking-water samples were collected from Seoul, Taejon, and Suwon. The samples were chlorinated (> 0.2 ppm) tap water supplied by water treatment plants to homes. Dissolved organic matter was extracted from 2000 l water by adsorption on XAD-2 resin in a glass column (3.5 cm in diameter and 25 cm in length) that had been cleaned prior to use by the method of Junk et al. [14]. The flow rate was about 1.5 l/min. Organic matter was eluted with 300 ml hexane:acetone 85:15 and 200 ml acetone (rate of 3–5 ml/min). The organic solvents were evaporated to a small volume at 40°C under reduced pressure and finally to dryness under a nitrogen stream (concentration factor, 2000). The dry residue was dissolved in 1 ml Tween 80 and then diluted in distilled water to a volume of 10 ml 200 l water equivalent/ml. Further dilutions were performed as necessary for the study. Tween 80 was used as a negative control. Samples were stored in a freezer at −20°C until use.

2.2. The Salmonella / microsome test

2.2.1. Tester strains

*S. typhimurium* TA98 and TA100 were obtained from Korean Food and Drug Administration. The strains were checked for histidine requirement, crystal violet sensitivity, and ampicillin resistance [11]. Background lawns were examined to check for cytotoxicity of the extracts and the possible presence of extraneous histidine.

2.2.2. Preparation of s9 mix

The S9 mix was prepared from livers of Sprague–Dawley rats pretreated with polychlorinated biphenyl mixture (Aroclor 1254) as described by Maron and Ames [10]. The S9 mix contained 10% S9 fraction, 8 μM MgCl₂, 33 μM KCl, 5 μM glucose-6-phosphate, 4 μM NADP, and 0.1 mM sodium phosphate buffer (pH 7.4).

2.2.3. Procedure

Mutagenicity was assayed by the method described by Maron and Ames [10]. In the presence or in the absence of S9 mix, mixtures of 100 μl water extract, 100 μl tester bacterial cultures [10 to 2] × 10⁹ cells/ml, and 2 ml soft agar supplemented with histidine and biotin were incubated for 25 min in the dark at 42°C. The mixtures were shaken briefly and immediately plated onto minimal medium. Cytotoxicity was determined by plating 100–200 cells with and without water extract onto the nutrient agar plates [10]. In the assays employing S9 fraction (16.3 mg/ml protein), 500 μl S9 mix was added per plate. All assays were carried out in triplicate. The plates were incubated in the dark for 48 h at 37°C, subsequently the revertants and surviving colonies were counted. Tween 80 was used as a negative control. The positive controls were 5 μg sodium azide for TA100 with or without S9 mix, 40 μg 2-aminofluorene for TA98 with S9 mix, and 10 μg 4-nitroquinoline-N-oxide for TA98 without S9 mix. The criteria for a positive response was a doubling of the background (spontaneous mutation) rate for at least one treatment and a dose-related response for a minimum of two treatments.

2.3. The micronucleus test

2.3.1. Animals

Male BDF1 mice were obtained from Department of Laboratory Animal Medicine, Seoul National University. The mice were approximately 7 weeks old and weighed 18–20 g at the time of dosing. They were acclimatized for at least 7 days, and then randomly distributed by weight into dosing groups. Groups of five mice were housed in polycarbonate
cages and given access to food (Samyang rodent pellet, Samyang, Korea) and water ad libitum. The animal facility was maintained at about 23°C with a 12 h light:dark cycle (0700–1900).

2.3.2. Preliminary test for bone marrow toxicity

In a preliminary test to determine bone marrow toxicity, doses (volume, 1 ml) of the sample extract equivalent to 100, 20, or 4 l of water were injected intraperitoneally (i.p.) into five (three male and two female) BDF1 mice. Tween 80 was used as negative control. The animals were sacrificed at 24 h post-injection (PD). Bone marrow smears and staining were performed as described below. A total of 1000 erythrocytes were counted from each animal. The ratio of polychromatic erythrocytes (PCE)/normochromatic erythrocytes (NCE) was calculated. No the PCE/NCE ratio differed significantly from that of the negative control in any treatment group, indicating that the water concentrates did not suppress bone marrow activity.

2.3.3. Procedure

On the basis of the result of the bone marrow toxicity test, we used three doses of the extract equivalent of 22, 11, and 3.7 l water for the micronucleus test. If a mouse weighing 20 g drinks 2 ml of water a day, the 22 l water would be the equivalent volume of what a human would consume in 30 years. Groups of 10 mice (five male and five female) were injected i.p. with 1 ml extract equivalent to 22, 11, or 3.7 l water. The mice were sacrificed 16, 24 or 48 h PI (highest dose) or 48 h PI (lowest and middle doses). The micronucleus test was conducted essentially as described by Schmid [15]. Bone marrow cells collected from femurs were fixed in methanol for 5 min, stained with Giemsa for 20 min or acridine orange [16] for 1–2 min, and evaluated at ×1000 by light and fluorescence microscopy. Five hundred PCEs were evaluated for each animal.

2.4. Statistical analysis

The Dunnet’s t-test or Student t-test were used to determine significant differences between the control group and the treated groups at the level of $p < 0.05$.

3. Results

3.1. The Salmonella / microsome test

In strain TA98 without S9 mix, the number of spontaneous revertant colonies per plate was 19 and the number of revertant colonies induced by 0.5 μg 4-nitroquinoline-N-oxide per plate (positive control) was 282. Drinking water extracts from all three cities showed positive responses in the Ames test, the sample from Seoul being the most mutagenic (Fig. 1a). In TA98 with mix, the spontaneous revertant number was 28 (Fig. 1b) and the number of revertant colonies of 40 μg 2-aminofluorene per plate (positive control) was 1422. Only the sample from Seoul
was mutagenic (Fig. 1b). Thus, metabolic activation significantly decreased the mutagenicity of the water extracts in strain TA 98.

In strain TA100, the drinking water extracts from all three cities showed mutagenic activity with and without S9 mix, the activity being greater without it (Fig. 2). The spontaneous revertants in strain TA100 without S9 mix and with S9 mix were 135 and 112, respectively (Fig. 2). The number of revertant colonies of 5 μg sodium azide per plate (positive control) was 1714. The water extract from Suwon had the most pronounced effect in the absence of S9 mix (Fig. 2a), whereas the water extract from Taejon had the most mutagenic activity in the presence of S9 mix (Fig. 2b).

![Fig. 2. Induction of revertants by drinking water extracts from three cities in Korea in S. typhimurium strain TA100 (a) without S9 mix and (b) with S9 mix.](image)

### Table 1

<table>
<thead>
<tr>
<th>Dose equivalent (1/mouse)</th>
<th>PI time (h)</th>
<th>Micronucleus frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seoul</td>
<td>Taejon</td>
</tr>
<tr>
<td>3.7</td>
<td>48</td>
<td>0.40 ± 0.84</td>
</tr>
<tr>
<td>11.0</td>
<td>48</td>
<td>0.40 ± 0.70</td>
</tr>
<tr>
<td>22.0</td>
<td>48</td>
<td>2.70 ± 1.258</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>0.70 ± 0.67</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>1.00 ± 1.05</td>
</tr>
</tbody>
</table>

Data represent the mean ± SD. Positive control (2 mg of mitomycin C/kg b.w.): 15.13 ± 3.40, negative control (Tween 80): 0.10 ± 0.32.

*The number of micronucleated polychromatic erythrocytes (PCEs) was calculated from 500 PCEs per animal.

**Significantly different from negative control group at p < 0.001.

***Significantly different from negative control group at p < 0.01.

****Significantly different from negative control group at p < 0.05.

### 3.2. The micronucleus test

No significant reduction in PCE/NCE ratios was caused by extracts of 100, 20, or 4 l water samples from the three cities, indicating that the extracts have no bone marrow toxicity. At the highest dose, water extracts from all three cities induced a significant increase of the micronucleus frequency (Table 1). The equivalent dose given as, five repeated exposures of 4.4 l water equivalent at 24 h intervals, however, did not increase the micronucleus frequency (data not shown). The time intervals required to increase the micronucleus frequency appear to be different between the samples. The waters from Seoul and Taejon induced the highest frequency at 48 h PI, whereas the water from Suwon induced the highest frequency at 16 h PI (Table 1).

### 4. Discussion

In general, there are three sources from which mutagenic chemicals in drinking water might arise: (1) natural products in the source water, (2) industrial and/or agricultural contamination of the source
water, or (3) products formed during water treatment and/or distribution [1,4]. The use of chlorine (to be over 0.2 ppm in tap water) as a disinfectant, which is practiced in Korea, may be at least partially responsible for the presence in drinking water of nonvolatile mutagens as well as THMs [17,18]. In this study, although we did not assay nonvolatile mutagens or THMs, we expected them to be present. Using the same tap water as we did, other investigators did not detect organic pesticides or heavy metals such as Cd, Hg, and Pb at the levels of micrograms per liter [19,20]. In addition, we did not detect mutagenic activity in the untreated water from the three treatment plants in a prior study with S. typhimurium TA98 and TA100 with and without S9 mix [21]. These findings together with the present result suggest that addition of chlorine are responsible for the mutagenicity of the treated water. These results are consistent with other reports [1,4].

The genotoxic activity of drinking water has been most frequently studied in bacteria, but results from mammalian cell assays are generally consistent with data from the bacterial assays [1,2,17]. However, chlorination products of water are highly mutagenic in bacteria, but negative or only marginally positive in in vivo micronucleus frequency with rodents [1,2,17,19]. In our study, intraperitoneal administration of organic extracts equivalent to 22 l water induced micronucleus formation in hemopoietic cells whereas five repeated exposures of one-fifth of the dose given at 24-h intervals did not. This finding suggests that there could be a threshold for the genotoxicity of the extracts, and a small amount of genotoxins in the drinking waters could be inactivated or detoxified by enzymes.

The drinking water extracts from all three cities showed mutagenic activity in TA98 without S9 mix and in TA100 with and without S9 mix. Strain TA98 detects frameshift mutations whereas TA100 detects base pair substitutions. In our study, TA100 was more sensitive than TA98, indicating that the organic water extracts might cause mainly point mutations. In addition, S9 mix caused a decrease in the mutagenicity of the drinking water extracts. This observation is consistent with other reports [18,22].

This study indicated that the mutagenicity of the drinking water in Seoul, Taejon, and Suwon was mainly due to chlorination of the native waters. However, the contamination of industrial and/or agricultural waste products and sewage could not be excluded. Strategies for drinking water treatment with respect to minimizing genotoxicity may include granular activated carbon filtration, chemical destruction, and the use of alternative means of disinfection such as ozonation, chlorine dioxide treatment, and chloramination, but such alternatives disinfect less effectively than chlorination. Therefore, the best way to improve drinking water quality is to protect source waters against contamination by agricultural and industrial waste and sewage.

Acknowledgements

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References

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