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〔Original Paper〕

Cytotoxicity of tap and first-class-river water in eastern Japan

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SUMMARY

Water was collected from the taps of houses in cities and from first-class rivers in eastern Japan, and organic compounds in the water were extracted and concentrated using an Oasis HLB 3-cc extraction cartridge. Human RSa cells were examined for assessing the cytotoxic effects of the concentrated compounds present in water by MTT assay. The viability of cells treated with tap water samples was found to be about 80-100% compared with 100% in cells treated with MilliQ water samples. The viability of cells treated with 17 river water samples was over 80% except for the sample from the Aganogawa river; water samples from Tamagawa and Arakawa rivers showed less than 70% viability in their middle and lower sections. The deterioration in water quality was particularly evident in April and July in Tamagawa river and in August and October in Edogawa river. Tests of the cytotoxic effects of water samples using human RSa cells may be a more comprehensive approach in evaluating biological effects of various factors in tap and river water and in unifying the criteria that apply to environmental water quality.

Key words: water quality, cytotoxicity, tap water

Ⅰ. Introduction

The supply of tap water in Japan depends largely on utilizing river water, and thus it is important for human health that good-quality river water is obtained. To evaluate water quality, the levels of at least 40 chemical compounds stipulated by the Water Works Law[1]are monitored during the process of tap water production. However, there are many more active compounds than this number in the environment generally and in river

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water in particular. Therefore, in addition to assessing the compounds present in the water, a method is required whereby the biological effects of compounds derived from river water components may be evaluated.

In the present study, we examined human RSa cells to assess the biological effects of environmental agents. The highly sensitive RSa cells were found to respond to lethal effects of ultraviolet radiation (mainly 254-nm wavelength, UVC)[2], 4-nitroquinoline 1-oxide (4NQO)[3], *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)[3], X-rays[4,5], and cadmium[6]. RSa cells are also appropriate for studying the molecular mechanisms underlying the action of environmental agents because variants derived from RSa cells can be easily established from cells mutagenized by ethyl methanesulfonate (EMS)[3,7]and from those transfected with plasmid vectors containing objective genes[8,9]. Thus, RSa cells can be utilized for evaluating the cytotoxic effects of compounds present in water.

Ⅱ. Materials and Methods

Cells and culture conditions

RSa cells were established from human embryo fibroblast cells doubly infected with Simian virus 40 and Rous sarcoma virus[10]; they have been characterized as having high susceptibility to environmental agents[2-6,11]. Cells were cultured with Eagle's minimal essential medium (Nissui, Tokyo, Japan) containing 10% (v/v) calf serum (Invitrogen, Carlsbad, CA, USA) at 37℃ in a humidified atmosphere containing 5% (v/v) CO_2.

Collection of environmental water

Tap water was collected from the faucets of city houses in Tokyo and Chiba Prefecture; about 5L water was allowed to flow prior to collection. River water was mainly collected upstream from the area where the water is supplied to the purification plants at first-class rivers in the northern area of Honshu (Japan) from 2003 to 2010. Except for Tamagawa, Tonegawa, and Arakawa rivers, water was collected upstream, not downstream, of the purification plants (Fig. 1). The river and tap water were collected in brown glass bottles, transported to our laboratory, and then stored at −20℃. Each water sample was allowed to thaw before tests were conducted.

Fig. 1 The locations where water samples were obtained in first-class rivers are indicated by "⊘".

Preparation of water samples

A wide spectrum of acidic, basic, and neutral organic compounds was extracted and concentrated from water collected using an Oasis HLB 3-cc extraction cartridge, according to the manufacturer's instructions. Briefly, 250 ml water was loaded into the cartridge, which had been preconditioned with methanol and Milli-Q water (Millipore, Tokyo, Japan); the cartridge was washed with 2.0 ml 5% methanol, and then compounds adsorbed to the cartridge were eluted with 2.0 ml methanol. The flow rate was maintained at 2 ml/min. Methanol was evaporated using a desiccator, and the dry residue was dissolved in 25 μl dimethyl

sulfoxide. The solution was used as the water sample.

Cell viability test

Cells were treated with and without water samples, as follows. Cells were seeded in each well of 96-well plates (5×10^3 cells/well) and cultured for 48 h. The activity of mitochondrial succinic dehydrogenase was measured by incubating the cells for 4 h in the presence of MTT (0.5 mg/ml) followed by measurement of absorbance at 570 nm with a reference wavelength of 655 nm, according to the method of Mosmann[12]. The viability of cells treated with Milli-Q water was taken to be 100% survival.

Statistical analysis

All experiments were repeated at least three times independently, and statistical analysis was performed using Student's *t*-test with Microsoft Office software (Excel 2003; Microsoft, Redmond, WA, USA).

Ⅲ. Results

The viability of RSa cells treated with tap water samples was over 80% in Tokyo and six prefectures (Fig. 2A). In addition, the viability was over 80% in Komazawa, Tokyo, and in Urayasu and Makuhari, Chiba Prefecture; however, the viability was under 80% in Hachioji, Tokyo, and in Kashiwa, Chiba Prefecture (Fig. 2B). Susceptibility of RSa cells to the cytotoxic effects of bisphenol A was examined by MTT assay: Fifty % viability was obtained at about 400 μM, while up to 80% viability was obtained at about 250 μM (Fig. 2C).

To estimate the safety of river water, the viability of RSa cells treated with the river water samples was tested. The viability after river water treatment was about 100% for

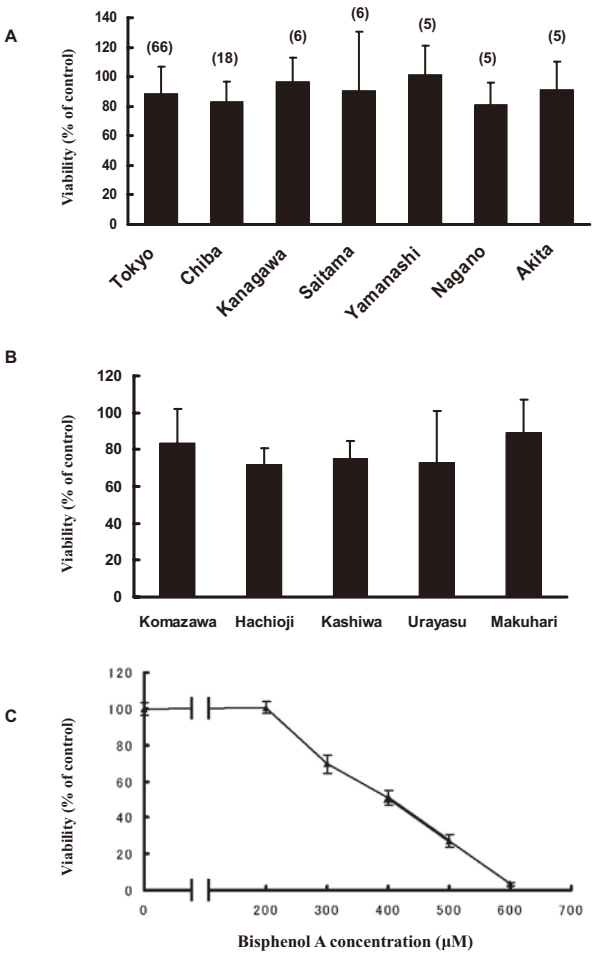

Fig. 2 Viability test of tap water samples in Tokyo and different prefectures with an indication of the numbers of faucets examined (A); and tap water samples obtained from houses in different cities (B); RSa cells treated with and without bisphenol A by MTT assay (C). More than five samples in Tokyo and each prefectural area (A) and three water samples in city (B) were collected at different times. The bars represent ±SD.

upper sections of Tamagawa river, though around 80% for middle and lower sections of the river (Fig. 3A). In similar fashion, Tonegawa and Arakawa samples showed 100-105% and 80-50% viability in upper and lower sections of the rivers, respectively (Figs. 3B and C).

Further, river water collected every month from 2004 to 2005 from Ozaku and Kanamachi, along the middle sections of the Tamagawa and Edogawa rivers, respectively, was tested. Decreased cell viability of under 70% was found

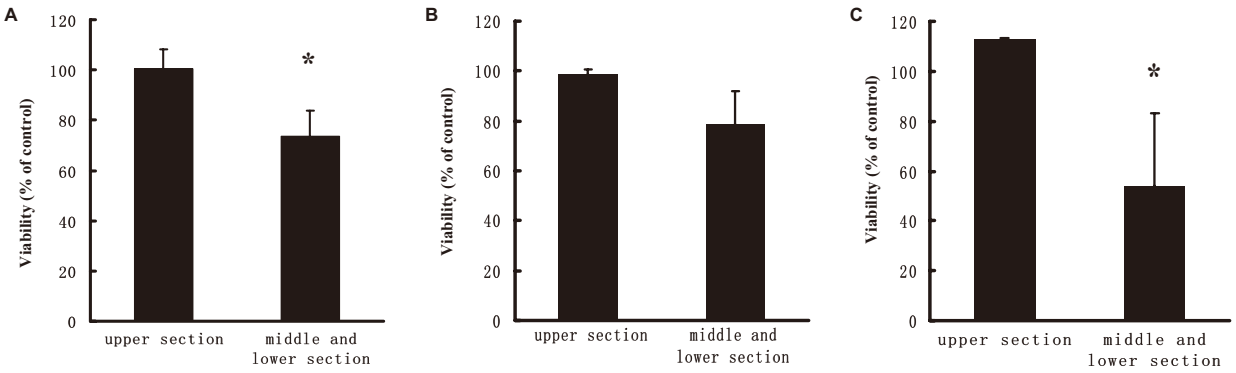

Fig. 3 Viability test of RSa cells treated with and without water samples from the Tamagawa (A), Tonegawa (B), and Arakawa (C) rivers. Bars represent ±SD. *, $P<0.05$ vs. upper section.

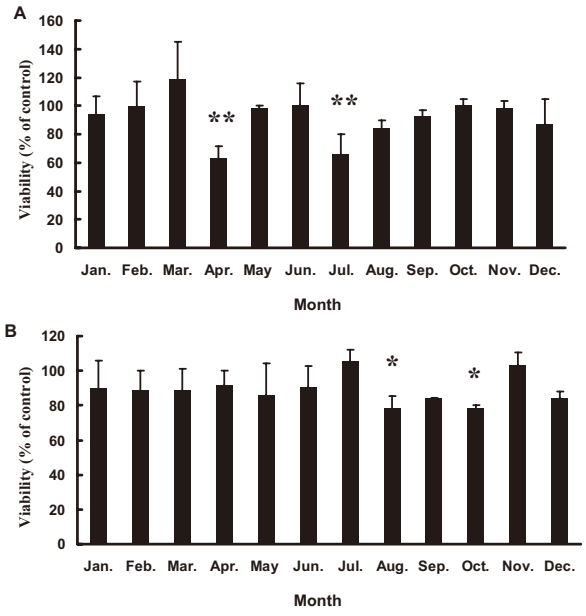

Fig. 4 Viability test of RSa cells treated with and without water samples from the Tamagawa (A) and Edogawa (B) rivers, which were collected in Ozaku and Kanamachi, respectively, every month for a year. Bars represent ±SD.*, $P<0.05$ vs. average, **, $P<0.005$ vs. average.

in April and July in Ozaku (Fig. 4A). Decreased cell viability of about 80% was also detected in August and October in Kanamachi (Fig. 4B).

Among the 7 first-class rivers in the Kanto and Koshinetsu regions (upstream of purification plants), treatment of RSa cells with water samples from Arakawa river resulted in about 75% viability; there was no obvious decrease in viability among the other six rivers (Fig. 5A). In the Tohoku region, 90-

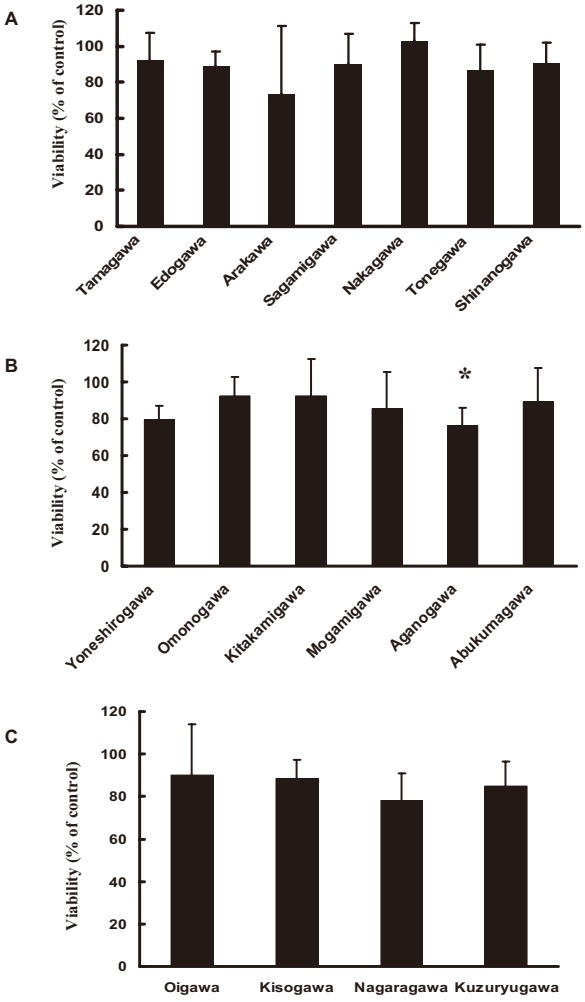

Fig. 5 Viability test of RSa cells treated with and without water samples from the first-class rivers in the Kanto and Koshinetsu region (A), Tohoku region (B), and Tokai, Hokuriku, and Chubu region (C). Bars represent ± SD. *, $P<0.05$ vs. average.

100% viability was detected in the three river samples; however, the viability was about 80% in samples from Yoneshirogawa, Mogamigawa, and Aganogawa rivers (Fig. 5B). Among four first-class rivers in the Tokai, Hokuriku, and Chubu regions, samples from Nagaragawa river showed about 80% viability, while those from the other three rivers showed over 80% viability (Fig. 5C).

Ⅳ. Discussion

In the present study, the viability of human RSa cells treated with tap water samples and water samples from 17 first-class rivers from eastern Japan was tested and found to be, respectively, about 80-100% (Fig. 2A and B) and over 70% (Fig. 5). Viability of 80-100% was detected in RSa cells treated with less than about 250 μM bisphenol A (Fig. 2C). We previously reported the mutagenicity of bisphenol A to be less than 10 μM in RSa cells [11]. Viability of 100% is required for tap water. However, the viability of human RSa cells treated with tap water samples was about 80-100%, or less than 80% in some cases. Therefore, it may be necessary to introduce methods that are able to purify water to a greater level than those currently adopted.

Treatment of cells with samples from five first-class rivers resulted in viability of about 80% or less (Fig. 5). In addition, samples from upper sections of Tonegawa and Arakawa rivers and those from the upper and middle sections of Tamagawa river conferred 80-100% viability; however, samples from middle and lower sections of the Tonegawa and Arakawa rivers and from the lower sections of the Tamagawa river resulted in less than 80% viability (Fig. 3). Many cities in the metropolitan Tokyo area are located around the middle and lower sections of these three rivers, notably around the lower sections of Tamagawa and Arakawa rivers. The lowering of water quality in these three rivers may be related to urban pollution, such as sewage.

After testing the viability every month for a year, it was found that the river water quality of Tamagawa and Edogawa rivers deteriorated in April and July (Fig. 4A) and also in August and October (Fig. 4B). These months correspond to times of active farming activity around the Ozaku and Kanamachi sampling sites[13,14]. Shioda et al. reported that toxic effects of river water on aquatic organisms were assessed and the samples collected of Tamagawa between March and August showed toxic effects[14]. They found the contamination of some pesticides [14]. And Ozaki et al. reported that biological activity and atmospheric temperature procured aggravation of water quality[15]. So there may have been some negative agrochemical and agricultural effects on the water quality in these two rivers.

In Japan, the criteria for water quality are based on the Water Works Law. Many countries around the world refer to the guidelines for drinking-water quality established by the World Health Organization. In the United States, the Safe Drinking Water Act was legislated by the Environmental Protection Agency. In the European Union, the Drinking Water Directive was introduced to ensure safe drinking water[1]. Even though many laws and directives have been established, there are differences among them. So the present study is constructive in helping to unify criteria with regard to water quality.

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要　旨

都市家庭用水道水および東日本地域の一級河川の水を採取し，水中の有機化合物をOasis HLB 3 ccカラムに吸着させ，その後，濃縮した。水に含まれる濃縮化合物の細胞毒性効果を評価するため，ヒトRSa細胞を用いたMTT法を行った。超純水で処理した細胞の生存率を100％とした場合，水道水サンプルの細胞生存率は約80％～100％であった。阿賀野川を除く17の河川水サンプルの細胞生存率は80％以上であった。また，多摩川と荒川の中下流域から得られた水サンプルは70％以下の生存率を示した。多摩川においては，４月と７月に採取されたサンプルで，江戸川においては８月と10月に得られたサンプルにおいて，特に水質悪化が認められた。培養ヒト細胞RSaを用い水サンプルの細胞毒性効果を調べることは，水道水や河川水中の様々な因子の生物学的影響を評価し，環境水の品質評価に応用することで，基準を統一することを可能とする包括的な手法である。

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ERRATUM

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