

Drinking water and liver cancer

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Abstract

AIM: To study the relationship between the mutagenicity of drinking water and incidence of liver cancer in high liver cancer incidence areas in Guangxi.

METHODS: A relationship between the mutagenicity of drinking water and incidence of liver cancer was studied in Fusui County, a high liver cancer incidence area in China. Thirty-two samples of different kinds of drinking water (13 samples of pond water, 3 samples of well water near the ponds, 5 samples of well water, 6 samples of river water and 5 samples of tap water) were tested with a micronuclear technique in the root tips of *Vicia faba*.

RESULTS: Among the 32 samples of different kinds of drinking water, 12 samples of pond water and 2 samples of well water near the ponds induced micronucleus frequencies on the root tips of *Vicia faba* to increase ($P < 0.01$), with the average micronucleus rate being 15.8% and 11.7%, respectively, while there was no difference between the micronucleus frequencies on the root tips of *Vicia faba* induced by well water (4.3%), river water (3.9%) or tap water (4.2%) and that on the control group ($P > 0.05$). Micronuclear effects on the root tips of *Vicia faba* in different kinds of drinking water were positively related to the incidence of liver cancer ($r = 0.86$, $P < 0.05$).

CONCLUSION: There were substances that caused chromosomal aberrations in the drinking pond water in high liver cancer incidence areas of Guangxi. Different kinds of drinking water were closely related to the incidence of liver cancer. Chemical mutagens in the water may be an important factor in the high incidence of human liver cancer.

Key words: Drinking water; Liver neoplasms; Legumes; Micronucleus tests

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INTRODUCTION

AFB₁, HBV infection and polluted drinking water are three important risk factors for the high incidence of liver cancer which have received extensive attention in the research of the pathogen for primary hepatic carcinoma (PHC). The epidemiological investigation has shown that there is a close relationship between polluted drinking water and the incidence of liver cancer. This relationship is especially common in the high liver cancer incidence area in Guangxi^[1] where we found that people who drank the pond water daily had a higher incidence of liver cancer than those who drank the well and river water^[2].

The purpose of our research was to observe the relationship between the drinking water and liver cancer using a micronuclear technique in the root tips of *Vicia faba* in order to provide a scientific basis for an epidemiological investigation.

MATERIALS AND METHODS

Spots and water sample collection

There are about 80 thousand people in Fusui County. We set up 32 spots in 23 villages according to the types of drinking water in the area and water samples were collected from each spot. The drinking water included pond water, well water near the ponds, well water, river water and tap water where Cl₂ (chlorine) had not been added. The water samples were put in plastic buckets separately and then kept at 10 °C for the experiment after being numbered. The *Vicia faba* is a green bean and the purely bred seed was provided by the Biology Department of Huazhong Teachers' University in China. The micronuclear background was 3%-5%. **The incidence of liver cancer**

Table 1 Micronuclear effects on the root tips of *Vicia faba* induced by different kinds of water

Water samples	The number of samples	MCN‰ ($\bar{x} \pm s$)	Induced effects
Pond water	13	15.8 ± 4.05 ^b	+
Well water by the ponds	3	11.7 ± 6.15	+
Well water	5	4.3 ± 0.74	-
River water	6	3.9 ± 0.87	-
Tap water	5	4.2 ± 0.85	-
Distilled water	12	4.5 ± 0.58	-
K ₂ Cr ₂ O ₇	12	25.1 ± 5.10 ^b	+

Each sample was compared with the distilled water (*t* test). ^b*P* < 0.01.

Table 2 Micronuclear effects on the root tips of *Vicia faba* induced by pond water

Spots	MCN‰ ($\bar{x} \pm s$)	Induced effects
Mumin	13.3 ± 1.24 ^b	+
Lingfan	16.3 ± 4.73 ^a	+
Bayang	15.3 ± 2.52 ^b	+
Liansui	18.0 ± 2.00 ^a	+
Ruilong	9.7 ± 0.58 ^a	+
Zhongyuan	15.3 ± 2.50 ^b	+
Busha	16.0 ± 1.60 ^a	+
Sairen	20.0 ± 1.63 ^a	+
Lailu	5.0 ± 1.00	-
Sanhe	8.7 ± 1.15 ^b	+
Dubang	21.7 ± 4.16 ^b	+
Jutun	19.3 ± 1.53 ^b	+
Tanlong	18.0 ± 2.16 ^b	+
Distilled water	4.4 ± 0.58	-
K ₂ Cr ₂ O ₇	25.0 ± 3.24 ^b	+

MCN of water from each spot was compared with distilled water (*t* test). ^b*P* < 0.01, ^a*P* < 0.05

Table 3 Micronuclear effects on the root tips of *Vicia faba* induced by well water by the ponds

Spots	MCN‰ ($\bar{x} \pm s$)	Induced effects
Tangan	17.7 ± 8.14 ^a	+
Mumin	3.0 ± 1.00	-
Jucheng	14.3 ± 3.05 ^a	+
distilled water	4.1 ± 0.25	-
K ₂ Cr ₂ O ₇	25.6 ± 5.20 ^b	+

MCN of water from each spot was compared with distilled water (*t* test), ^b*P* < 0.01, ^a*P* < 0.05.

was the annual rate per 100000 derived from the reports from the Fusui Cancer Institute from 1973 to 1987. All the cases in the reports had been investigated and checked by researchers in this specific field.

Micronuclear experiments on the root tips of *Vicia faba*

The tests were carried out according to the method, procedure and requirements of Degress and other reports^[3,4]. Routine germinating, handling, pruning, fixing and hydrolysis were conducted. After that, we took 3 root tips from each sample, observed 1000 cells under the super microscope, calculated the number of the micronucleus cells (MCN) and the micronucleus rate (MCN %). **Statistical analysis** of MCN% was carried out on the tested and control group. Distilled water was used as a negative control and K₂Cr₂O₇ (3.4 × 10⁻⁶ mol/L) as a positive control by each assay.

RESULTS

Table 1 shows the micronuclear effects on the root tips of *Vicia faba* induced by different kinds of water samples. Tests of 32 samples of different water samples showed that micronucleus frequencies of *Vicia faba* root tips induced by pond water were the highest (*P* < 0.01), next was well water near the ponds (*P* < 0.05), while there was no difference between the micronucleus frequencies induced by well water, river water or tap water and the negative controls (*P* > 0.05).

Table 4 Micronuclear effects on the root tips of *Vicia faba* induced by well water

Spots	MCN‰ ($\bar{x} \pm s$)	Induced effects
Ruilong	5.00 ± 1.00 ^a	-
Sairen	3.30 ± 0.47 ^a	-
Dubang	4.67 ± 1.15 ^a	-
Tangan	3.85 ± 0.57 ^a	-
Zhongyuan	4.51 ± 0.51 ^a	-
Distilled water	4.33 ± 0.52	-
K ₂ Cr ₂ O ₇	24.9 ± 3.10 ^b	+

MCN of water from each spot was compared with distilled water (*t* test), ^b*P* < 0.01, ^a*P* < 0.05.

Table 5 Micronuclear effects on the root tips of *Vicia faba* induced by river water

Spots	MCN‰ ($\bar{x} \pm s$)	Induced effects
Longtun	5.33 ± 0.57	-
Nale	4.00 ± 1.00	-
Wangzhuang	4.10 ± 0.85	-
Liuqiao	3.66 ± 0.75	-
Funong	5.67 ± 0.50	-
Longtou	4.66 ± 1.10	-
Distilled water	4.23 ± 0.52	-
K ₂ Cr ₂ O ₇	25.70 ± 2.30 ^b	+

MCN of water from each spot was compared with distilled water (*t* test), ^b*P* < 0.01.

Table 6 Micronuclear effects on the root tips of *Vicia faba* induced by tap water

Spots	MCN‰ ($\bar{x} \pm s$)	Induced effects
Changping	3.33 ± 0.57	-
Zhongdong	4.33 ± 0.55	-
Nongyang	5.00 ± 1.00	-
Tangan	4.12 ± 0.87	-
Yiqiao	3.80 ± 0.51	-
Distilled water	4.30 ± 0.50	-
K ₂ Cr ₂ O ₇	25.71 ± 2.91 ^b	+

^b*P* < 0.01.

Table 7 Relationship between different kinds of drinking water and the incidence of liver cancer

Type of drinking water	Incidence of liver cancer (/100 thousand)	RR
Pond water	82.56 ^b	2.34
Well water by the ponds	75.85 ^b	2.17
Well water	63.12 ^a	1.80
River water	45.69 ^a	1.31
Tap water	35.01	1.00

Comparison between the incidence of liver cancer induced by different kinds of water and that induced by tap water, ^b*P* < 0.01, ^a*P* < 0.05

Table 2 shows the micronuclear effects on the root tips of *Vicia faba* induced by pond water. Tests of 13 pond water samples showed that 12 samples of water induced increased micronucleus frequencies on the root tips of *Vicia faba*, with a positive rate of 92.3% and the average micronucleus rate between 8.7%-21.7%.

Table 3 shows the micronuclear effects on the root tips of *Vicia faba* induced by well water by the ponds. Tests of 3 water samples showed that 2 samples induced increased micronucleus frequencies on the root tips of *Vicia faba*.

Table 4, Table 5 and Table 6 show the micronuclear effects on the root tips of *Vicia faba* induced by well water, river water and tap water separately. There was no difference between the micronucleus frequencies induced by these water samples and the controls (*P* > 0.05).

Table 7 shows the relationship between the different kinds of drinking water and the incidence of liver cancer. 40000 people who drank pond water had a higher incidence of liver cancer than the 40000 who drank well water by the ponds, well water and river

Table 8 Relationship between micronuclear effects induced by different kinds of drinking water and the incidence of liver cancer

Type of drinking water	MCN‰ ($\bar{x} \pm s$)	Incidence of liver cancer (/100 thousand)
Pond water	15.8 ± 4.05	82.56
Well water by the ponds	11.7 ± 6.15	75.85
Well water	4.3 ± 0.74	63.12
River water	3.9 ± 0.87	45.69
Tap water	4.2 ± 0.85	35.01

MCN induced by different kinds of drinking water was compared with the incidence of liver cancer, ($r = 0.86, P < 0.05$).

water, while those who drank tap water had the lowest incidence of liver cancer. There is a marked difference between the incidence of liver cancer induced by different kinds of drinking water other than tap water and that induced by tap water.

Table 8 shows the relationship between micronuclear effects on the root tips of *Vicia faba* induced by different kinds of drinking water and the incidence of liver cancer. The micronuclear effects induced by pond water samples was strong, so the people who drank pond water had a higher incidence of liver cancer, while the incidence of liver cancer among those who drank well water, river water and tap water was low. Micronuclear effects on the root tips of *Vicia faba* induced by the substances in different kinds of drinking water coincided with the incidence of liver cancer ($r = 0.86, P < 0.05$).

Comparing the micronuclear effects induced by different pond water samples with the incidence of liver cancer among the local people, we found that the micronuclear effects induced by pond water agreed with the changing incidence of liver cancer on the whole. However, the micronuclear effects induced by different pond water samples changed greatly because of the varying degrees of pollution of the pond water and its interrelation with the incidence of liver cancer was not significant ($r = 0.53, P > 0.05$).

DISCUSSION

The relationship between polluted drinking water and liver cancer is difficult to understand and has still not been elucidated due to the limited experimental means. Many scholars have noticed the common phenomenon that polluted drinking water will cause a high incidence of liver cancer. In our study we found that 12 samples of pond water and 2 samples of well water by the ponds in 32 samples of different kinds of drinking water collected in Fusui County induced micronucleus frequencies on the root tips of *Vicia faba* to increase, indicating that chemical mutagens polluted the drinking pond water in Fusui County. This kind of substance can distort chromosomes in plant cells and has mutagenicity. It suggested that ingesting these mutagens and/or carcinogens may increase the latent danger for

people to develop liver cancer and provided experimental evidence for the etiology of liver cancer.

Research shows that drinking highly polluted surface water, water with added Cl_2 and water with a high concentration of $CHCl_3$ will increase the risk of liver cancer. The risk may come from many mutagens and/or carcinogens which have interrelated and cooperative roles in the polluted drinking water^[5]. In our research, we found mutagenic substances in the drinking pond water of Fusui County. The people who drink polluted pond water have the highest incidence of liver cancer, which is 2.34 times as much as the incidence of liver cancer in those who drink tap water. The degree of the micronuclear effects induced by different kinds of water coincided with the incidence of liver cancer ($r = 0.86, P < 0.05$), that is to say, people who drink highly polluted water and water with a strong micronuclear effect have a high incidence of liver cancer. Although a single chemical mutagen and/or carcinogenic substance may have a very low concentration in the polluted water at a PPb or PPM level, this will harm the DNA and reach a carcinogenic threshold for animals and humans drinking it long term. Frequent exposure to the carcinogenic environment with AFB₁ and mycotoxins and so on^[6] may be another important risk factor for the high incidence of liver cancer.

There has been no definitive final conclusion about the cause of liver cancer to date but many scholars think that liver cancer is caused by the complementary effects of multiple carcinogenic factors. Therefore, preventing hepatitis, reducing intake of AFB₁ and drinking hygienic water^[7] are basic strategies to prevent liver cancer. Our research shows that there may be some specific carcinogens in the drinking water of high liver cancer incidence areas. So, the incidence of liver cancer will be lowered by controlling and preventing pollution of water with all kinds of mutagens and/or carcinogens. It is predicted that liver cancer will be the first cancer that man can prevent. Certainly we can.

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