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氚水的致癌效应研究

STUDIES ON CARCINOGENIC EFFECT OF TRITIATED WATER

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摘　要

氚水的致癌效应研究工作包括两部分。第一部分为体外细胞转化实验，使CHL－1细胞受氚水照射24～96 h，比活度为9.25×10^{5}～3.5×10^{6} Bq/ml，累积剂量为0.055～0.88 Gy。并以^{137}Cs γ射线为参考射线，以恶性转化率为终点求得氚水相对于γ射线的RBE值为1.6。第二部分观察了大鼠长期（1.5 a）饮用氚水，其比活度为2.22×10^{5}，1.11×10^{5} Bq/ml，对照组饮用自来水。结果表明，大剂量组的肿瘤总发病率和恶性肿瘤的发病率与小剂量组和对照组相比在统计学上有明显差异。

STUDIES ON CARCINOGENIC EFFECT OF TRITIATED WATER

(*In English*)

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ABSTRACT

Studies on carcinogenic effect of tritiated water is introduced in two parts. The first part is an *in vitro* study in which CHL-1 cells were exposed to tritiated water ($9.25\times10^5\sim3.5\times10^6$ Bq/ml) for 24～96 h and the accumulated dose was from 0.055 to 0.88 Gy. In order to estimate RBE of tritium for malignant transformation in CHL-1 cells, the induction of malignant transformation in CHL-1 cells by exposure to gamma rays of ^{137}Cs was tested. Based on the transformation rates, the RBE of tritium for malignant transformation in CHL-1 cells was estimated to be 1.6. The second part is an *in vivo* study. In the study, rats were fed with tritiated water (2.22×10^5 and 1.11×10^5 Bq/ml) for 1.5 a. Rats in control group were fed with tap water. Results showed that in the statistics, the differences in the total tumor incidence and malignant tumor incidence between high and low dose rate groups and control groups were remarkably significant.

Except natural tritium , a large quantity of tritium is involved in nuclear energy operations, being a significant by-product of nuclear fission and fusion. The hazard of tritium in the environment is becoming of increasing concern. Tritium generated by these sources is ultimately converted into tritiated water, which becomes widely disseminated in the biosphere, offering the potential of human exposure. Recently several of international conferences of biological effects of nuclide-tritium released from nuclear industries have been held [1]. Many reports have drawn attention of researchers of its low level toxicity on the different experiments. Among the biological effects of tritium, its carcinogonesis is the most important in assessment of health risk of tritiated water to human. In this paper, the results of both *in vitro* and *in vivo* studies on carcinogonic effect of HTO were reported.

1 THE *IN VITRO* STUDIES ON

1.1 Induction of Malignant Transformation in CHL-1 Cells by Exposure to Tritiated Water

A number of studies have been carried out on lethal, cytogenetic, and mutagenic effects of HTO in mammalian cells [4,5]. The induction of mutation and chromatosome aberrations is indirect measures of the carcinogenic potency of physical or chemical agents. The current investigation, therefore, was undertaken to examine the abillity of protracted exposure to HTO to induce the maligant transformation of CHL-1 cells *in vitro*.

1.2 Materials and Methods

(1) Tritiated water (HTO) was purchased from the China Institute of Atomic Energy in 1 ml aliquots of 3.7×10^{10} Bq. The HTO was diluted gradually with the bi-distilled water to the desired activity and checked by a liquid scintillation counter (Backman) .

(2) Culture medium CHL-1 cells were grown in RPMI 1640 medium (Nissui , Japan) with 10% newborn calf serum and kanamycin sulphate (50 IU/ml) .

(3) Chinese Hamster lung cell A CHL-1 cell strain establihed by Dr. Utakoji T. (Tokyo Oncologic Institute) was used in these studies. This strain of cell is a kind of epithelioid cells and can creep on the wall of sealed bottle.

(4) Method for culture of cells Cells were cultured in nutrient medium and exposed to HTO or ^{137}Cs Gamma rays after a 24 h incubation of 37℃ when they have creeped on the bottle wall.

(5) Exposure of cells to HTO Some cells were exposed to HTO at doses of 0, 9.2×10^5, 1.85×10^6 and 3.7×10^6 Bq/ml, respectively, for 24, 48, 72 and 96 h. For these cells, the absorbed dose from HTO was estimated based on the specific activity in the culture medium, following Kapoor [3]. The accumulated doses were shown in Table 1.

(6) Expsure to ^{137}Cs Gamma rays Gamma-ray irradiation of cells was carried out with ^{137}Cs at a dose rate of 0.359 Gy/d at 37° C for 24, 48, 72 and 96 h. So that accumulated doses were 0.359, 0.718, 1.08 and 1.44 Gy, respectively.

(7) Methods for calculation of malignant transformation rate At the end of exposure period, the cell cultures were washed twice with PBS balanced salt solution and then suspended by 0.25% trypsin solution. These suspended cells were reseeded into replicate bottles with 40×60 mm^2 square in fresh medium at low density and incubated at 37° C for 7～9 d. Then, the cells were washed with PBS solution and were fixed and stained with 0.5% solution of crystal violet. The transformed colonies were observed under microscope. A cluster with 50 or more cells is considered as a colony. The normal colony is constituted by monolayer cells. The transformed cells show a loss of contact inhibition and pile up randomly (Fig. 1). Transformed and normal colonies were counted [5,6].

Fig 1 Colonies of CHL-1, ×40
a —Colony of normal cells;
b —Colony of transformed cells

Fig. 2 Transformed cells under cell-culture microscope, ×600

(8) Mrophological observation.

(a) Alive cells were observed under a cell-culture-microscope;

(b) Histological method Before seeding, a cover glass was put into the bottle to allow cells to creep onto it. After incubation, the cover glass was taken out and prepared for pathological study. Cells were fixed with 95% alcohol and stained with hamatoxylin and eosin.

with hamatoxylin and eosin.

(9) Test on the transplant ability of transformed cells To test the transplant ability of transformed cells, treated cells were injected subcutaneously into irradiated (7.83 Gy) 3-week old Swiss mice (5×10^5 cells/mouse). Mice were kept for one month until animals died. Autopsy was made to search tumor at the sites of injection. Tumor, if any, was taken out and histopathologically examined to identify transformed cells in it.

1.3 Results

1.3.1 Malignant transformation rate

These results clearly indicate that protracted exposure to HTO will induce the malignant transformation of CHl-1 cells. The frequencies of malignant transformation were shown in Table 1. For CHL-1 cells, the frequency of spontaneous transformation was 0.89%. After treatment by radiation, the frequencies of trasformation were increased obviously. The differences in frequency of cell transformation were found statistically to be very significant ($P<0.01$) between cells exposed to HTO or gamma rays and controls.

The data of malignant transformation form HTO-exposed cells can be fitted to a linear regression equation

$$Y=2.9984+13.51X \qquad (r=0.9460)$$

where X=accumulated doses of cells exposed to HTO (Gy);

Y=malignant transformation rate (%).

The frequencies of malignant transformation of cells exposed to ^{137}Cs gamma rays were shown in Table 2. The data of malignant transformation from these cells also can be fitted to a linear regression equation

$$Y=0.2548+8.6172\,X \qquad (r=0.9864)$$

Table 1 Malignant Transformation in CHL-1 Cells by Tritiated Water

Term irrad. (h)	Activity of HTO (10^4 Bq/ml)	Cumulative doses (Gy)	Colonies counted	No. of transformed colonies	Transfor mation rates (%)
24	0.925	0.055	10092	331	3.28±1.22
	1.850	0.110	8076	382	4.7±1.53
	3.700	0.220	11813	705	5.97±1.05
48	0.925	0.110	9796	473	4.83±1.04
	1.850	0.220	9089	530	5.83±0.87
	3.700	0.440	10223	1133	11.10±1.51
72	0.925	0.165	9124	471	5.16±1.63

(续表 1)

	1.850	0.330	6755	464	8.97±0.94
	3.700	0.660	9931	1184	11.9±1.97
96	0.925	0.220	8142	564	6.93±1.67
	1.850	0.440	6160	645	10.50±2.19
	3.700	0.880	9889	1290	13.04±2.10
	0	0	6353	57	0.89±0.42

Table 2 Malignant Transformation in CHL-1 Cells Exposed to ^{137}Cs Gamma Rays

Duration of exposure (h)	Cumulative doses (Gy)	Colonies counted	No. of transfromed colonies	Transformation rates (%)
24	0.359	9054	235	2.59±1.06
48	0.718	4835	328	6.78±2.16
72	1.080	10102	863	8.54±1.99
96	1.440	11176	1496	13.4±1.99
0	0	6353	57	0.89±0.42

These findings show that for CHL-1 cells there is better dose-effect relationship in malignant transformation for both HTO and gamma rays. For HTO beta rays, the relative biological effectiveness (RBE) for malignant transformation of CHL-1 cells can be calculated to be 1.6 , based on the ratio of slops of both above mentioned linear regressions.

1.3.2 Morphological observation

Some pathological changes were fornd in exposed cells.

(1) Under the cell-culture-microscope, obvious morphological changes of some cells, such as markedly irregular outlines and randomly piled-up (Fig. 2), may be seen.

(2) For the transformed cells, aniskaryosis, thicker nuclear membrane than normal, aggregated chromatin in nucleuses and obvious nuclei may be found histopathologically (Fig. 3)

1.3.3 Test for transplant ability of transformed cells

After injection, tumours in size of about 2～3 mm^3 were found in some mice injected with irradiated CHL-1 cells, and the histopathological study showed that cells in these tumors were identical with injected transformed CHL-1 cells (Fig. 4) . No tumour was found in mice injected with non-irradiated cells. It is shown that the transformed cells have the transplant ability and the normal cells are without such ability.

Fig 3 Transformed cells after exposure to HTO
H. & E., ×1200

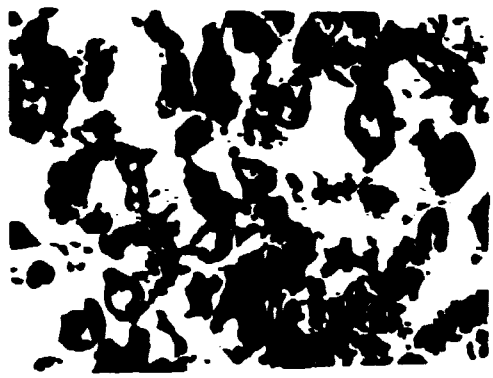

Fig 4 Histopathological section of tumor formed from transformed cells
H. & E., ×600

1.4 Discussion

It is well known that ionizing radiation induces DNA damage and the error in DNA repair results in the synthesis of abnormal proteins and mitosis delay which may lead to gene mutation or cell death. The appearance of these effects depends on doses and species of animals. For example, the lethal dose is 1.5～6 Gy of beta rays for the BALB/3T3 cell [7] and for the V79-79 cells it is 3～8 Gy (doss rate is 1.35 Gy/min) of gamma rays [8]. The doses for both radiations used in this experiment are lower than the above-mentioned lethal doses.

Little [7] reported that exposure to HTO at low-dose rate (25～100 μg/ml) can induce malignant transformation in BALB/3T3 cell line. The cell line and the methods used are different between Little's report and this paper. However, the results of both show that HTO can induce malignant transformation in mentioned cells at low dose rates.

2 THE *IN VIVO* STUDIES ON

2.1 The Carcinogenic Effect in Rats Drinking Tritiated Water for Long Term

As showed in the previous experiments, tritiated water at low doses can induce malignant transformation in CHL-1 cells. This study was performed for further examination of the carcinogenic effect of protracted exposure of animals to low dose of tritiated water.

2.2 Materials and Methods

(1) Tritiated water with a specific concentration of 3.7×10^{10} Bq/ml was

obtained from the China Institute of Atomic Energy, Beijing. It was diluted furth.. to desired concentration with distilled water and checked on a liquid scintillation counter (Packard Co., USA) for determining its activity.

(2) Animals Spraque Dewle strain rats were procured from the Experimental Animal Center, the Chinses Academy of Proventive Medicine, Beijing. 136 rats were intoxieated were used in this experiment.

(3) Intoxication S.D. rats at 40-days old by intraperitoneal injection of tritiated water. For 50 rats (high dose rate dose rate group), tritiated water was injected into them so that the activity in their body water reachs approximately to $1.11\times^{5}$ Bq/ml and for 46 rate (low dose rate group), to 5.55×10^{4} Bq/ml. At the same time, 40 rats were injected with distilled water and served as control group. The amount of body water for the rat was considerted to be 62% of its body weight. After injection, rate for high dose rate group were allowed to drink *ad libitum* tritiated water with a specific concentration of 2.22×10^{5} Bq/ml throughout 547 d and those for low dose rate group to drink tritiated water with a specific concentration of 1.11×10^{5} Bq/ml, but rats for control group only drank tap water. Animals for experimantal groups were maintainted and bred in a special laboratory with ventilator and animals for control group in a well-breezy room. All animals were fed with the food prepared for rats.

(4) Dosimetry When HTO has entered into body, it is uniformly distributed in various body fluids, including body water, and can not be concentrated by kidney. From this, it can be considered that concentration of HTO in body water is the same as in urine [9,10]. Based on the results of monitoring of tritium in urine, it is thought that the concentration of tritium in body water is approximally a half of that in drinking water when it is in a halance. There is a relationship as follows:

$$I=0.49\,W \tag{1}$$

where I is the concentration of tritium in body water and W is the concentration of tritium in drinking water. In addition, it is also well known that the activity of tritium in wet tissues is equal to the sum of activity for tritium in body water and that for incorporated tritium in tissue. According to the methods cited in [10, 11], the absorbed dose (D) can be estimated by the following equation:

$$\begin{aligned} D &= 0.21\,It \\ &= 0.21\times0.49\,Wt \\ &= 0.103\,Wt \end{aligned} \tag{2}$$

where t is the duration (in days) of drinking tritiated water.

(5) Observation During the observation period, the tested animals were examined every day for their general status and periodically for body weight, WBC count and differential count of WBC, and development of tumors. If animal died in the course of experiment the autopsy must be carried out. At the end of experiment, all animals were sacrified for autopsy. In the autopsy for both died and sacrified animals, the weight and size were measured for all important organs, such as heart, liver, spleen, lung, kidney and, in sometimes, brain, and for tumors, if any, and from them, samples were taken for histopathological study. From the organ samples, slides were prepared by a routine pathological technique after staining with Harris haematoxylin and eosin. They are observed under optical microscope for determining the cause of death and the types of tumor.

2.3 Results and Discussion

2.3.1 Incidence of tumors

After drinking tritiated water for a certain term, some tumors were developed in tested rats. The results were shown in Table 3. From Table 3, it can be seen that for high dose rate group, the total tumor incidence was 56% and the incidence of malignant tumors was 34%. For low dose rate group, these incidences were 30.4% and 6.5%, respectively, and for control group, these parameters were 22.5% and 7.5%, respectively. For high dose rate group, both the total tumor incidence and the incidence of malignant tumors are significantly higher than those for low dose rate group and control group ($P<0.05$ and $P<0.01$).

In the high dose rate group, more rats (20%) with two types of tumors were observed. Among them, 6% of animals had brought two malignant tumors, 12% of animals—one malignant tumor and one benign tumor, and other 2%—two benign tumors. In low dose rate group, rats with two types of tumours were less (8.6%) tham those in the high dose rate group and all of them were brought two benign tumors. In control group, no rat was found to bring two types of tumors. Tumors for rats in high dose rate group appeared about 200 d earlier than that for rats in low dose rate group.

2.3.2 Type of tumors

As mentioned above, both malignant and benign tumors may be induced for rats drinking tritiated water in the long term. Based on the results of histological study, 60% of tumors observed in rats were benign and 40% of them were malignant. The distribution of tumour type in these groups was shown in Table 4.

2.3.3 Activity of tritium in urine

In order to monitor the level of tritium in body water, the urine samples were collected from some animals at various time during this experiment for determining the specific concentration of tritium in urine. The results showed that at the first

Table 3 Incidence of Tumor in Rats after Long-termly Drinking Tritiated Water

Dose rate (mGy/d)	Cumulated doses (Gy)	No. of animals	Animals with tumors			
			No	%	with malig. tumors No.	with malig. tumors %
6.18	3.35 (1.54~3.35)	50	28	56	17	34
3.09	1.72 (1.39~1.72)	46	14	30.4	3	6.5
0	0	40	9	22.5	3	7.5

Table 4 Types of Tumor in Rats after Long-termly Drinking Tritiated Water

Types of Tumor	6.18 mGy/d			3.09 mGy/d			Control		
	NO.	M	F	No.	M	F	No.	M	F
Fibrocarcinoma	5	3	2	1			1	3	3
Adenocarcinoma in breast	6		6	2	1	1			
Leukemia	2	1	1						
Rhabdomyosarcoma	2	2							
Adenocarcinoma in lung	2	2							
Malignant lymphoma	1	1							
Squamous-carcinoma	1	1							
Undifferentiated carcioma	1	1							
	20			3			3		
Fibroma	7	4	3	3	2	1	1	1	
Adenoma in breast	6		6	8		8	3		3
Adenofibroma in breast	4		4	4		4	2	1	1
Adenoma in lung	1	1							
	18	5	13	15	2	13	6	2	4
Total	38			18			9		

day after intoxication of tritium, the level of tritium in body water was approximately close to the expected value, that is, the specific concentration of tritium in body water was $1.0\sim1.07\times10^5$ Bq/ml for high dose rate group and $4.92\sim5.51\times10^4$ Bq/ml for low dose rate group. At the 64th and 138th days, the values were

1.06×10^5 Bq/ml for high dose rate group and 5.25×10^4 Bq/ml for low dese rate group, that indicated the level of tritium in body water for tested animals was constant and at a balance status throughout this experiment. Based on these results, it can be considered that the method for intoxication used here is suitable for induction of carcinogenic effect in rats.

When tritiated water entered into the body it was uniformly ditributed in the body fluids without target behaviour, therefore, the content of tritiated water in tissues depends upon their water content. All the tumors observed in this experiment were tumors from soft tissues, such as fibrocarcinoma, adenocarcinoma, leukemia, malignant lymphoma, fibroma and adenoma of breast, it may be due to high water content in soft tissues. No tumour was developed in the tissues with less water content, such as bone and fat.

These results suggest that the incidence of tumors in rats can be significantly increased by drinking tritiated water in the long term at the dose range used in this experiment.

3 CONCLUSION

For a long time, it was believed that tritium is a radionuclide with low toxicity. However, form the observations, that tritiated water can induce malignant transformation in CHL-1 cells and a high incidence of tumors in rats drinking tritiated water for long term. It indicates that for tritium, there is carcinogenic effect which is considered as a non-threshold stochastic effect. Therefore, it is suggested that the carcinogenicity of tritium must be considered in the development of radiation protection standard for tritium.

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