Carcinogenic Effects of Tritiated Water (HTO) in Mice: In Comparison to those of Neutrons and Gamma-rays

TOSHIO SEYAMA¹, OSAMU YAMAMOTO², AIKO KINOMURA³ AND
KENJIRO YOKORO¹

¹ Radiation Effects Research Foundation

² Faculty of Science, Hiroshima University

³ Research Institute for Nuclear Medicine and Biology, Hiroshima University

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A series of experiments was carried out using female (C57BL/6N×C3H/He)F₁ mice (BCF₁) to assess the carcinogenic effect of tritiated water (HTO) following a preliminary experiment in which the acute effect of HTO was studied in young female mice of C57BL/6L strain in an attempt to gain basic information on the long-term experiment, and the results were compared with those of fission spectrum neutrons and gamma-rays. The obtained findings are summarized as follows. 1) C57BL/6N mice receiving a single intraperitoneal (i.p.) injection of HTO, 7.4×10^8 Bq (20 mCi) or more, died of bone marrow failure within 20 days. 2) In long-term experiment, BCF₁ mice given 1.4×10^8 Bq to 5.6×10^8 Bq developed solid tumors in a variety of tissues but with no obvious dose dependency. 3) The fractionated exposure of 7.4×10^8 Bq (1.9×10^8 each, at 7-day intervals) was highly effective in induction of malignant T-cell lymphomas (85%) with a shorter latency than that of a single exposure (15%). 4) A protracted irradiation of 0.27 Gy of ¹³⁷Cs, designed to simulate the decreasing absorption rate with time after a single i.p. injection of an equivalent dose of HTO resulted in a drastic reduction in induction rate of ovarian tumors as compared with that of an acute ⁶⁰Co gamma irradiation. 5) The carcinogenic potential of HTO, given as a single i.p. injection, was quite similar to that of acute ⁶⁰Co gamma ray irradiation at both 2.7 Gy and 0.27 Gy levels. On the other hand, the effect of HTO was slightly higher than that of protracted gamma-ray irradiation. 6) ²⁵²Cf fission neutrons was found to be more potent in tumor induction than gamma-rays or HTO beta-rays under the present experimental conditions.

INTRODUCTION

It is important to assess the long-term effects, especially of carcinogenic effect of tritium beta-rays because of the possible leak of tritium to our environment when nuclear fusion reaction is utilized as the energy source in near future. Once tritium is released into our environment, it is rapidly oxidized to tritiated water (HTO) which will be widely dispersed.

There is abundant information on the somatic, cytogenetic and genetic effects of HTO in mammals.¹⁻⁴⁾ The influences of dose and dose-rate in carcinogenesis induced by neutrons, x-rays and ⁶⁰Co gamma-rays in mice have been investigated in detail by Upton, et al,⁵⁾

while only fragmental information is available as to carcinogenic effect of HTO. The present study was undertaken to evaluate the carcinogenic effect of HTO in mice and to compare the findings with those of fission neutrons and gamma-rays.

MATERIALS AND METHODS

Animals: Female mice of C57BL/6N and of C57BL/6N×C3H/He) F_1 (BCF $_1$) were purchased from Charles River Japan. They were irradiated at age of 7–8 weeks. The mice were kept in an air-conditioned room ($24^{\circ}\text{C}\pm 2^{\circ}\text{C}$) after neutron and gamma-ray irradiations. HTO-treated mice were maintained in a closed animal chamber,⁶⁾ temperature of which was also kept at $24^{\circ}\text{C}\pm 2^{\circ}\text{C}$.

HTO administration: HTO with an activity of 1.0 Ci/ml (Amersham, UK) was diluted with saline to variously assigned concentrations. A half ml of HTO in saline was intraperitoneally injected to each mouse.

Fission neutron irradiation: Mice were placed individually in acrylite tube and exposed to neutron emitted from a ^{252}Cf source⁷⁾. A total of 2.7 Gy/min and SCD of 11.9 cm.

Gamma-ray irradiations: For acute irradiation, mice were exposed to either 2.7 Gy or 0.27 Gy from a ^{60}Co source at a dose-rate of 0.47 Gy/min and SCD of 80 cm. For subacute irradiation, mice were exposed to ^{137}Cs at a dose-rate of 0.14×10^{-2} Gy/min and SCD of 100 cm. For chronic exposure to gamma-rays, a ^{137}Cs source was utilized. This device was constructed in our Institute⁸⁾ as a tritium simulator. It was operated at a starting dose-rate of 0.53×10^{-3} Gy/min for a total dose of 2.7 Gy and 0.59×10^{-4} Gy/min for 0.27 Gy, respectively. For both doses, it took 16.6 days and 12.2 days, respectively.

Observations: To detect carcinogenic effect of respective radiation, irradiated mice were observed for 750 days after irradiation. The mice with overt tumors or moribund were sacrificed and subjected to a complete autopsy after hematological examination. Paraffine-embedded tissue sections were stained with Hematoxylin-Eosin for pathological diagnosis.

RESULTS

I. Effects of a single intraperitoneal injection of HTO

Acute and subacute effects: Seven-week-old female C57BL/6N mice received a single intraperitoneal injection of various doses of HTO; 80 mCi (29.6×10^8 Bq), 40 mCi (14.8×10^8 Bq), 20 mCi (7.4×10^8 Bq), 10 mCi (3.7×10^8 Bq) and 0 mCi (0 Bq) per mouse. The survival rate and change in the body weight of each group are shown in Fig. 1. All mice receiving HTO of more than 20 mCi died within 20 days after injections, while there was no mortality among mice receiving 10 mCi up to 28 days and beyond. The body weight of mice destined to die decreased progressively with no recovery, while those of mice receiving 10 mCi recovered their weights following a transient decrease. The thymus and spleen weights and peripheral blood cell counts decreased rapidly in a dose dependent manner (data not

Fig. 1. Survival curves and body weight changes.
Seven-week-old mice were injected intraperitoneally with various concentrations of HTO. Each group consists of 20 female C57BL/6N mice.

shown).

Long-term effects: Female BCF₁ mice were given a single intraperitoneal injection of 3.75 mCi (1.4×10^8 Bq), 7.5 mCi (2.8×10^8 Bq), 15 mCi (5.6×10^8 Bq) and 20 mCi (7.4×10^8 Bq) of HTO per mouse. The total absorption doses were about 1.97 Gy, 3.95 Gy, 7.90 Gy and 10.53 Gy, respectively, according to generally accepted radiation dose conversion from Bq to Gy.⁹⁾ Fig. 2 shows the cumulative incidence of tumor development in the 4 experimental groups. The incidence of tumors at 400 days after HTO injection were 4%, 8%, 18% and 24% in mice given 3.75 mCi, 7.5 mCi, 15 mCi and 20 mCi of HTO per mouse, respectively. After 500 days following HTO injection, however, the incidences were not much different among the groups, indicating that the lowest dose level, namely, 3.75 mCi, was already enough to elicit a high incidence of tumors. The difference in tumor response among groups appeared to be represented by the earlier onset of tumors in higher dose groups. Tumor incidence in non-irradiated control mice was low in this experiment.

Fig. 2. Cumulative incidence of tumors among mice receiving single intraperitoneal injection of HTO. Seven-week-old mice were injected intraperitoneally with various concentrations of HTO. Each group consists of 60 female (C57BL/6N×C3H/He)Fd21 mice.

II. Induction of T-cell lymphoma/leukemia by fractionated intraperitoneal injection of HTO.

Four weekly injections of 5.0 mCi each, total of 20 mCi, or 3.75 mCi each, total of 15 mCi, also resulted in a high yield of tumors. However, the prominent feature of such treatment was the early and high yield of T-cell lymphomas initiating in the thymus, especially in the 20 mCi group and the incidence reached to 80%, and all lymphomas completed to appear within 220 days. The finding is quite similar to that observed in mice after the fractionated total-body irradiation of X-rays or gamma-rays.¹⁰⁾ The incidence of lymphomas was 25% in mice given 3.75 mCi×4 times. However, the cumulative incidence of tumor development was 76% in this group, which was not much different from that in 5.0 mCi×4 group. It was shown that the difference in total dose of HTO had a prominent effect in lymphomagenesis when the dose was fractionated. Solid tumors started to appear after 270 days at which time the development of lymphoma is almost over regardless of single or fractionated injection (data not shown). These results indicated that there was no difference

Cumulative Incidence of Leukemia among Mice receiving either Single or Fractionated i.p. Administration of HTO

Fig. 3. Cumulative incidence of lymphoma among mice receiving either single or fractionated intraperitoneal injection of HTO.

Seven-week-old mice were injected intraperitoneally with 0.5 ml of various concentrations of HTO. Each group consists of 60 female (C57BL/6N×C3H/He) F_1 mice.

in carcinogenic effect between single and fractionated injection of HTO except lymphoma productivity.

III. Comparison of carcinogenic effect of HTO with those of fission neutrons and gamma-rays.

Carcinogenic effect of radiations with different quality was assessed in female (C57BL/6N×C3H/He) F_1 mice given either 2.7 Gy or 0.27 Gy of ^{252}Cf fission neutrons, ^{60}Co or ^{137}Cs gamma-rays and HTO beta-rays (1.9×10^8 Bq and 1.9×10^7 Bq equivalent to 2.7 Gy and 0.27 Gy, respectively).

In mice given 2.7 Gy of respective radiation, the cumulative tumor incidences at 500 days after irradiations were about 70% in neutron group, 35% in acute ^{60}Co gamma-rays, 30% in subacute ^{137}Cs gamma-rays (with a constant dose-rate), 25% in HTO beta-rays and 10% in protracted ^{137}Cs gamma-rays simulated to HTO absorption curve, and days to reach 50% of tumor incidence were 480, 590, 560, 580 and 650 days post irradiation, respectively. It was also evident that the cumulative tumor incidence increased rapidly in neutron irradiated

Fig. 4. Cumulative incidence of tumors among mice receiving an equal dose of radiation of different qualities (2.7 Gy).

Seven-week-old mice were injected intraperitoneally with HTO or irradiated with an equal dose (2.7 Gy) of radiations; ^{252}Cf fission neutrons, ^{60}Co gamma-rays and ^{137}Cs gamma rays. Each group consists of 60 female (C57RL/6N $\times$ C3H/He) F_1 mice.

fix = constant subacute irradiation

sim = protracted irradiation which simulated the decreasing dose rate after HTO injection.

group and much slowly in protracted gamma-ray irradiated group (HTO simulation by ^{137}Cs gamma-rays). But, the cumulative tumor incidences eventually reached 83–95%, and did now show much difference each other after a long-term observation (Fig. 4).

The similar results were obtained after 0.27 Gy of irradiation (Fig. 5). In this case, however, the onset of tumor development was delayed as compared with that of 2.7 Gy irradiated cases except neutron exposed group in which increase in the cumulative tumor incidence was earlier than those in other three groups, viz, acute gamma, HTO and protracted gamma-ray irradiation. The tumor incidence in non-irradiated control mice accelerated from about 650 days and reached to 60%. The majority of tumors developed among these mice were so-called "reticulum cell neoplasm (RCN)" which forms tumorous growths in the spleen, liver, uterus and lymph nodes without showing leukemic pictures in the peripheral blood. This spontaneously occurring tumor has been known to be characteristic among C57BL-related mouse strains.¹¹⁾ There was no remarkable difference in tumor incidence between 0.27 Gy and 2.7 Gy irradiated groups if the observation period was prolonged up to 750 days after irradiations.

It was found that a single irradiation of 2.7 Gy and 0.27 Gy of neutrons and gamma-rays, and also a single intraperitoneal injection of HTO were not lymphomagenic in BCF₁ mice even though fractionated injection of 20 mCi HTO was highly lymphomagenic. The frequency of lymphoma development in all experimental groups except 0.27 Gy HTO group

Fig. 5. Cumulative incidence of tumors among mice receiving an equal dose of radiations of different qualities (0.27 Gy).

Seven-week-old mice were injected intraperitoneally with HTO or irradiated with an equal dose (0.27 Gy) or radiations; ^{252}Cf fission neutrons, ^{60}Co gamma-rays and ^{137}Cs gamma-rays. Each group consists of 60 female (C57RL/6N $\times$ C3H/He) F_1 mice.

sim: protracted irradiation which simulated the decreasing dose rate after HTO injection.

was not different from that of non-irradiated control group. Although patterns of solid tumor development appeared roughly similar among mice exposed to different radiations, certain differences were noticed. For example, neutrons induced tumors of wider tissue distribution, viz., tumors in mammary gland, pituitary, liver, pancreatic islet and osteosarcomas were rather conspicuous at dose level of 2.7 Gy. Lung tumor incidence was higher among mice receiving HTO injection than that of protracted ^{137}Cs gamma-rays at both 0.27 Gy and 2.7 Gy levels (Tables 1 and 2). Frequency of ovarian tumor development was significantly lower in mice exposed to 0.27 Gy of protracted ^{137}Cs gamma irradiation than that of mice receiving 0.27 Gy of other radiations (Table 2). Tumor incidence in ovary, pituitary, liver and Harderian gland of mice irradiated with 0.27 Gy of neutrons was as high as that of mice receiving 2.7 Gy. While development of multi-tumors was not rare among irradiated mice, it was more frequently observed in neutron irradiated mice (data now shown).

DISCUSSION

A prominent carcinogenic effect of fission neutrons has been reported in certain experimental systems.¹²⁻¹⁵ In the present studies in mice, we showed again as in the rat mammary tumor system¹⁶, a greater carcinogenic effect of neutrons than that of HTO beta-rays and

Table 1. Tumor development in mice receiving an equal dose of radiation of different qualities (2.7 Gy). Seven-week-old mice were injected intraperitoneally with HTO or irradiated with an equal dose (2.7 Gy) of radiations; ^{252}Cf fission neutrons, ^{60}Co gamma-rays and ^{137}Cs gamma-rays. Each group consists of 60 female (C57BL/6N×C3H/He)F₁ mice.

a, b, c, d, e and f in the upper column indicate experimental groups: a, c, e; A single acute irradiation, b; A single injection of HTO, d; protracted irradiation of ^{137}Cs gamma-rays.

Numbers in the parentheses are % of tumor incidence per original number of mice in each group. a, b, c, d, e or f on shoulder shows that the incidence is statistically different from indicated group(s) either at $p < 0.05$ (*) or $p < 0.01$ by χ^2 test.

Table 1. Tumor Development in Female (C57BL/6N×C3H/He)F₁ Mice given an Equal Dose of Radiation of Different Quality (2.72 Gy)

Organ	Fission neutrons (^{252}Cf) ^a	Beta-rays (HTO) ^b	Gamma-rays (^{60}Co) ^c	Gamma-rays (^{137}Cs) ^d	Gamma-rays (^{137}Cs) ^e	Control ^f
Ovary	38 (62.3) ^f	43 (71.7) ^f	41 (68.3) ^f	45 (75.0) ^f	44 (75.9) ^f	1 (1.7)
Pituitary	33 (54.1) ^{b,c,e,f}	5 (8.3) ^{a,c,e*}	19 (31.7) ^{a,b,f*}	29 (48.3) ^{e,f}	15 (25.9) ^{a,b*d}	8 (13.3)
Harderian gland	10 (16.4) ^{f*}	6 (10.0)	7 (11.7)	5 (8.3)	7 (12.1)	2 (3.3)
Liver	24 (39.3) ^{b,c,d,e,f}	5 (8.3) ^a	8 (13.3) ^{a,f*}	5 (8.3) ^a	7 (12.1) ^a	2 (3.3)
Lung	3 (4.9)	8 (13.3) ^{e*}	6 (10.0)	9 (15.0) ^e	1 (1.7) ^{b*,d}	4 (6.7)
Mammary gland	16 (26.2) ^{b,c,d,e,f}	0 ^{a,d*}	2 (3.3) ^a	4 (6.6) ^{a,b*}	0 ^a	0
Uterus & Vagina	2 (3.3)	0	1 (1.7)	0	1 (1.7)	1 (1.7)
Adrenal gland	3 (4.9)	1 (1.7)	0	1 (1.7)	0	0
Bone	5 (8.2) ^{b*,d*}	0 ^{a*}	1 (1.7)	0 ^{a*}	2 (3.4)	1 (1.7)
Pancreas	8 (13.1) ^{b*,c*}	0 ^{a*}	1 (1.7) ^{a*}	1 (1.7)	0	0
Stomach	2 (3.3)	1 (1.7)	1 (1.7)	1 (1.7)	0	0
Skin	0	0	0	0	1 (1.7)	0
Thyroid	3 (4.9)	1 (1.7)	1 (1.7)	0	1 (1.7)	1 (1.7)
Soft tissue	1 (1.6)	1 (1.7)	5 (8.3)	5 (8.3)	2 (3.4)	2 (3.3)
Others						
RCN	3 (4.9) ^{d*,f}	1 (1.7) ^{c*,d,f}	8 (13.3) ^{b*,c}	11 (18.3) ^{a*,b,c}	0 ^{c,d}	15 (25)
Hemangiosarcoma	0	0	0	0	0	
Lipoma	4 (6.6) ^{b*,d,e*}	0 ^{a*,c*,e}	6 (10.0) ^{b*,d*}	16 (26.7) ^{a,c*,f}	12 (20.7) ^{a*,b,f}	0
Leukemia	2 (3.3)	6 (10.0)	7 (11.7)	4 (6.6)	7 (12.1)	1 (1.7)
						4 (6.7)

a,c,e: A single irradiation.

b: A single injection of HTO.

d: Protracted irradiation, simulated to HTO absorption curve.

Number in parenthesis are % of tumor per original mice.

a, b, c, d, e or f on shoulder shows that difference from indicated group is significant at $p < 0.05$ *, or $p < 0.01$ by χ^2 test.

Table 2. Tumor development in mice receiving an equal dose of radiations of different qualities (0.27 Gy). Seven-week-old mice were injected intraperitoneally with 0.5 ml of HTO or irradiated with an equal dose (0.27 Gy) of radiations; ^{252}Cf fission neutrons, ^{60}Co gamma-rays, ^{137}Cs gamma-rays. Each group consists of 60 female (C57BL/6N $\times$ C3H/He)F₁ mice.

a, b, c, d, and e in the upper column indicate experimental groups: a, c; A single acute irradiation, b; A single injection of HTO, d; protracted irradiation of ^{137}Cs gamma-rays.

Numbers in the parentheses are % of tumor incidence per original number of mice in each group.

a, b, c, d or e on shoulder of number shows that the incidence is statistically different from indicated group(s) either at $p < 0.05$ (*) or $p < 0.01$ by χ^2 test.

Table 2. Tumor Development in Female (C57BL/6N $\times$ C3H/He)F₁ Mice given an Equal Dose of Radiation of Different Quality (0.27 Gy)

Organ	Fission neutrons (^{252}Cf) ^a	Beta-rays (HTO) ^b	Gamma-rays (^{60}Co) ^c	Gamma-rays (^{137}Cs) ^d	Control ^e
Ovary	29 (46) ^{c*,d,e}	20 (33.3) ^{c,d,e}	42 (72.4) ^{a*,b,d,e}	4 (6.2) ^{a,b,c}	1 (1.7)
Pituitary	18 (28.6) ^{c*,e*}	12 (20.0)	7 (12.1)	10 (15.4)	8 (13.3)
Harderian gland	11 (17.5) ^{c,d,e}	5 (8.3)	1 (1.7) ^a	2 (3.1) ^a	2 (3.3)
Liver	8 (12.7) ^{b*,c*,e*}	2 (3.3) ^{a*}	1 (1.7) ^{a*,d*}	7 (10.8) ^{c*}	2 (3.3)
Lung	6 (9.5)	10 (16.7) ^{d*}	9 (15.5)	3 (4.6) ^{b*}	4 (6.7)
Mammary gland	4 (6.3) ^{c*}	5 (8.3) ^{c*}	2 (3.4)	1 (1.5)	0
Uterus & Vagina	4 (6.3)	5 (8.3)	2 (3.4)	3 & 1	1 (1.7)
Adrenal gland	2 (3.2)	2 (3.3)	1 (1.7)	0	0
Bone	0	2 (3.3)	1 (1.7)	0	1 (1.7)
Pancreas	0	0	1 (1.7)	0	0
Stomach	0	0	1 (1.7)	0	0
Skin	0	0	0	1 (1.5)	0
Thyroid	0	1 (1.7)	0	0	1 (1.7)
Soft tissue	3 (4.8)	5 (8.3)	2 (3.4)	3 (4.6)	2 (3.3)
Others					
RCN	10 (15.9)	5 (8.3) ^{c*}	11 (19.0)	9 (13.8)	15 (25.0)
Hemangiosarcoma	2 (3.2)	2 (3.3)	0	3 (4.6)	0
Lipoma	12 (19.0) ^e	9 (15) ^e	11 (19.0) ^e	6 (9.2)	1 (1.7)
Leukemia	9 (14.2)	15 (25.0) ^{d*,e}	10 (17.2)	6 (9.2) ^{b*}	4 (6.7)

^{a,c,*}: A single irradiation.

^b: A single injection of HTO.

^d: Protracted irradiation, simulated to HTO absorption curve.

Number in parenthesis are % of tumor per original mice.

a, b, c, d or e on shoulder of number shows that difference from indicated group is significant at $p < 0.05$, or $p < 0.01$ by χ^2 test.

^{60}Co or ^{137}Cs gamma-rays, which was represented by the earlier onset of tumor development in wider spectrum of tissues, and larger numbers of mice bearing multiple tumors as compared to other two sorts of radiations.

There has been little information as to the carcinogenic effect of HTO beta-rays in rodents. Cahill et al.^{17,18)} reported effect of HTO in mother rats exposed to different doses

of HTO on day 0 of pregnancy (total calculated doses were 0.044, 0.44, 2.2 and 4.4 Gy, respectively). The incidence of leukemia in mother rats was increased in 4.4 Gy group, incidence of mammary tumor elevated in 2.2 and 4.4 Gy groups, and shortening of life-span was observed in 0.44, 2.2 and 4.4 Gy groups. Among offspring of those mothers, there was no evidence of increased tumor rate or life-span shortening in 0.044 and 0.44 groups, while the frequency of mammary tumor development was decreased and life-span was shortened in 4.4 Gy group. In these studies, no effort was made to estimate the RBE of HTO. Gragtmans et al¹⁹⁾ studied effect of HTO in induction of mammary tumors in rats, and they estimated the RBE of HTO to be about 1.1–1.3 as compared with protracted total-body X-irradiation. However, the effect of acute total-body X-irradiation was slightly higher than that of HTO.

In the present experiments, a special study was carried out, in which mice were exposed to a protracted irradiation of ¹³⁷Cs gamma-rays with decreasing dose-rate to simulate dose absorption of HTO after its single i.p. injection. Only such a condition allows us to compare carcinogenic potential of HTO with that of gamma-rays. It was found that acute or subacute gamma-ray irradiation results in an equivalent level of carcinogenicity, while a protracted gamma-ray irradiation was less carcinogenic at 2.7 Gy (Fig. 4). Thus, these facts may suggest that the RBE of HTO on the carcinogenicity being slightly higher than unity, the RBE of HTO at 500 days after 2.7 Gy irradiation was about 2.5 times greater as compared to protracted gamma-ray irradiation.

We are the first to report a high efficiency of the fractionated i.p. injections of HTO in induction of T-cell lymphomas initiating in the thymus. The similar finding was first reported by Kaplan et al¹⁰⁾ several decades ago, in which a high frequency of T-cell lymphoma development was found in C57BL mice given the fractionated X-irradiations. They suggested the etiological involvement of lymphomagenic retrovirus (Radiation Leukemia Virus) in radiation-induced lymphomagenesis.²⁰⁾ However, our recent study indicated that the retrovirus is not necessarily involved.²¹⁾ More recently, the mutational activation of the ras gene family is indicated in radiation- and chemically-induced mouse T-cell lymphomagenesis.²²⁾ We have been observing an interesting finding in another experiment being underway in this laboratory, in which mice are kept on the drinking water containing HTO of various concentrations. Although the study is not completed yet, a high incidence, up to 75%, of T-cell lymphoma with a short latency is observed in mice given drinking water containing 0.25 mCi/ml of HTO.²³⁾ This is also a novel finding, and the virological and molecular studies to explore the etiological mechanism is a next subject of interest.

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