The Effects of Tritiated Water on the Bone Marrow Cells in Mice

TAKEHIKO TSUCHIYA1, TOSHIYUKI NORIMURA1, MIYUKI NIKAIDO1, HIROYO KAKIHARA1, HISAO YAMAMOTO2 and SATORU HATAKEYAMA2

Department of Radiation Biology and Health1 and Radioisotope Research Center2, University of Occupational and Environmental Health, Japan
1-1 Iseigaoka Yahatanishiku Kitakyushushi, 807, Japan

(Received June 17, 1988)
(Revised version, accepted August 9, 1988)

INTRODUCTION

With the development of the nuclear fusion reactor, the biological effects of tritium in humans need to be clarified. There are not many reports of in vivo investigations in mammals. Brues et al.1) first conducted a mortality study on mice administered a single injection of tritiated water (HTO). Furchner2) determined the RBE of tritium beta radiation using the 30-day mortality of mice after a single injection of HTO. Recently, Dobson et al.3,4), reported the effects of 14 days' continuous administration of HTO on mouse oocytes and Carsten et al.5) reported the effects of chronic exposure of HTO on bone marrow in mice.

The acute effects of tritium on hematopoietic tissue in human may be the most important in accidental exposure to tritium. Thus the acute effects of a single administration of HTO on bone marrow in mice were investigated in this study.
In many reports, the absorbed dose of HTO in organs is estimated from tritium content in body fluid. This method may not show the exact absorbed dose in the organ. In the present study the activity of tritium in the organ was measured directly using a sample oxidizer and the absorbed dose was calculated from the activity of tritium in the organ. The number of nucleated cells, CFUs, CFUc and CFUf in the bone marrow were determined to evaluate the effects of tritium on hematopoietic tissue. Furthermore, RBE of tritium was estimated by comparison with the effect of $^{60}$Co radiation at comparative dose rates.

**MATERIALS AND METHODS**

**Animals**

B6C3F$_1$ female mice, 10-12 weeks of age, were used. The mice injected with HTO were bred in a special cabinet for this study in an air-conditioned room as were the other mice. Each mouse was housed separately in a special small cage and fed commercial pellet chow and water ad libitum.

**HTO injection**

The mice were divided into four groups. Those of the first three groups were each singly injected with one ml of 4, 8 and 12 mCi of HTO into the peritoneal cavity and the mice of the last group were injected with one ml of distilled water in the same manner. These mice were sacrificed 2 days after injection and the tibiae and femora were removed and used for analysis.

**Absorbed dose calculation from beta radiation of HTO in bone marrow**

In our previous experiments, mice were sacrificed at different intervals after injection of HTO (500 $\mu$Ci/mouse), femora and tibiae were removed and the activity of tritium was measured using a sample oxidizer and liquid scintillation counter. The decay curves were obtained from activity of HTO in the bone marrow, and the effective half-life was 2.38 days.

If one assumes that the tritium is distributed uniformly throughout the tissue, the absorbed dose of bone marrow may be calculated in a conventional manner as follows:

$$d_{\beta} = \frac{E_{\beta} \cdot C \cdot k}{3.7 \times 10^4 \times 86400 \cdot E_{\beta} \cdot C}$$

$$= \frac{6.24 \times 10^{12}}{5.12 \times 10^{-4} \cdot E_{\beta} \cdot C \text{ (Gy/day)}}$$

$$= 2.92 \times 10^{-3} \cdot C \text{ (Gy/day)}$$

where $d_{\beta}$ is the absorbed dose rate in Gy per day, $E_{\beta}$ is the average energy of $^3$H beta rays (5.7keV), C is the specific activity at time 0 ($\mu$Ci/g wet tissue), k is a constant relating kilo-electron volts per gram to Gy ($1\text{Gy} = 6.24 \times 10^{12} \text{ keV/g}$), $3.7 \times 10^4 = \text{disintegrations per } \mu\text{Ci per second}$, and 86400 = seconds per day. Thus, cumulative absorbed dose ($D_2$) until day 2 is

$$D_2 = \int_0^2 d_{\beta} (t) \cdot dt$$

$$= 2.92 \times 10^{-3} \int_0^2 C(t) \cdot dt$$

$$= 4.21 \times 10^{-3} \cdot C \cdot T \left(1-e^{-0.693 \times 2/T}\right) \text{ Gy}$$
where $C(t)$ is the specific activity at time $t$ ($C(t) = C \cdot e^{-0.693t/T}$), and $T$ is the effective half-life of $^3$H in the bone marrow (days). When $C = 34.3 \ \mu$Ci/g wet tissue after administration of 500 $\mu$Ci per mouse, and $T = 2.38$ days, then,

$$D = 0.152 \text{ Gy}$$

Assuming the absorbed doses are proportional to the total amount of HTO administered, injection of 4, 8 and 12 mCi per mouse was calculated to give 1.21, 2.42 and 3.63 Gy, respectively, during the two day post-administration period. The details of this method will be published in a separate paper.

$^{60}$Co irradiation

The mice were divided into four groups: The first three groups were exposed to whole body irradiation of $^{60}$Co $\gamma$-rays for 2 days by Tandem apparatus. Total radiation dose in the first, second and third groups of mice was 1.25, 2.5 and 3.75 Gy, respectively. These radiation doses were adjusted by changing of the distance of mice from $^{60}$Co source. The dose rate and integral dose were measured by Ionex (2500/3) and Harshaw TLD-100 dosimeter, respectively. The dose rate of the first, second and third groups was 45.8 mR/min, 91.5 mR/min and 137.3 mR/min, respectively. The fourth group was a sham-irradiated control.

Analysis of CFUs, CFUc and CFUf

The nucleated cell suspension of bone marrow was prepared from the tibiae and femora and these cells were used for colony forming assay.

i) Nucleated cell number

The mice were sacrificed by chloroform and heart puncture 2 days after HTO injection or after irradiation of $^{60}$Co $\gamma$-rays for 2 days. The white blood cells in peripheral blood were counted after heart puncture, and the femora and tibiae were removed from mice and bone marrow cells were suspended in RPMI medium and the number counted by hemocytometer.

ii) Colony assay

a) CFUs: The cell concentration of bone marrow cell suspension was adjusted to $5 \times 10^5$/$\mu$l (control or low dose irradiated group) or $1 \times 10^6$/$\mu$l (high dose irradiated group) by RPMI medium. The adjusted marrow cell suspension (0.2ml) was intravenously injected into mice which had been exposed to whole body X-irradiation of 7 Gy a day before injection. After 8 days the mice were sacrificed, their spleens removed and fixed by Bouin's solution, and the number of colonies on the spleens counted.

b) CFUc: The bone marrow cells were suspended in medium, diluted to the appropriate concentration and plated into one ml McCoy's 5A medium supplemented with 40% horse serum, 500 units CSF (colony stimulating factor-"CHUGAI") and 0.3% agar (final concentration) in 35-mm diameter dishes. The cultures were incubated for 7 days at 37°C in a humidified atmosphere of 5% CO$_2$ in air and the colonies (discrete groups of more than 50 cells) were scored using an inverted microscope.

c) CFUf: The bone marrow cells were plated at cloning densities (densities adjusted to obtain 20-80 colonies per dish) into 5 ml McCoy's 5A medium supplemented with 10% horse serum, 10% fetal calf serum and 10% L-cell conditioned medium in 60-mm diameter dishes, and incubated
for 14 days under the same conditions as for CFUc. The visible colonies were counted after Giemsa staining.

RESULTS

The cell survival was calculated from the colony forming efficiencies of the irradiated cells compared to that of the unirradiated controls. The surviving fraction was plotted as a function of dose of tritium beta rays and $^{60}$Co $\gamma$-rays, and regression lines and the values of Do and $D_{37}$ were obtained by the least squares method.

i) Nucleated bone marrow cells

The experimental data and regression lines for the number of nucleated bone marrow cells are shown in Fig. 1. The experimental data for HTO and $^{60}$Co are similar and the regression curves agree approximately with each other. The $D_{37}$ and Do values for HTO were 3.73 Gy and 3.70 Gy, and 3.40 Gy and 3.40 Gy for $^{60}$Co gamma radiation, respectively, and the RBE value of tritium beta rays relative to $^{60}$Co gamma rays is 0.91 at $D_{37}$ level.

ii) CFUs (colony forming unit-spleen)

The experimental data and regression lines for CFUs are shown in Fig. 2. As shown in Fig. 2, results for tritium and $^{60}$Co are similar, and the regression curves agree approximately with

![Graph](image_url)

Fig. 1. Dose-response relationship in nucleated bone marrow cells for 2 days exposure to HTO and $^{60}$Co gamma radiation.
each other. The $D_{37}$ and $D_0$ values for HTO were 1.72 Gy and 1.72 Gy, and 1.88 Gy and 1.80 Gy for $^{60}$Co gamma radiation, respectively. The RBE value of tritium beta rays is 1.09 at $D_{37}$ level.

iii) CFUc (colony forming unit-culture)

The experimental data and regression lines for CFUc are shown in Fig. 3. As shown, the same dose of HTO is much more effective than that of $^{60}$Co gamma radiation. The $D_{37}$ and $D_0$ values for HTO were 2.18 Gy and 2.03 Gy, and 3.30 Gy and 3.30 Gy for $^{60}$Co gamma radiation, respectively. The RBE value of tritium is 1.51 at $D_{37}$ level.

iv) CFUf (fibroblastoid colony forming unit)

The experimental data are similar in two lower dose points but the highest dose point of $^{60}$Co gamma rays was not obtained, as shown in Fig. 4. Thus the regression curve could not be obtained for the $^{60}$Co group.

![Dose-response relationship in CFUs for 2 days exposure to HTO and $^{60}$Co gamma radiation.](image-url)
DISCUSSION

The acute effect of tritium on bone marrow in mice was studied using the following four biological endpoints: the numbers of nucleated cells, CFUs, CFUc and CFUf. The dose-response curves were derived from the observed data, and the RBE values of tritium beta rays relative to $^{60}$Co gamma rays were obtained for each biological endpoint. The RBE value of tritium for numbers of nucleated bone marrow cells, CFUs, and CFUc was calculated to be 0.91, 1.09, and 1.51, respectively, for 2 days' exposure of HTO and $^{60}$Co gamma radiation. The effect of tritium on bone marrow death of mice was first investigated by Brues et al.\textsuperscript{1}) and the 30-day mean lethal dose of HTO for mice was shown to be about 1 mCi per g body weight and the estimated radiation dosage to be about 1000 rep in the first 12 days after a single injection. However, the RBE value of tritium was not shown in their paper. Thereafter, Furchner\textsuperscript{2}) determined the RBE of tritium beta rays by using 30-day mortality of mice after a single injection of HTO. The reference radiation was $^{60}$Co radiation given at an exponentially decreasing rate reflecting the effective half life of HTO during an 8-day period. He showed the radiation dose of LD50/30 for $^{60}$Co and tritium to be 1350 and 804 rad, respectively, and the RBE value of tritium beta rays relative to $^{60}$Co gamma rays was 1.72.
Furthermore, in the report of Storer et al.\textsuperscript{6}, the RBE value of tritium relative to radium gamma radiation was 1.32 and 1.52 using the endpoints of atrophy of mouse spleen and thymus, respectively, for depression of $^{59}$Fe uptake by red cells in rats, they obtained an RBE value of 1.64 relative to $^{60}$Co gamma radiation.

Recently, Kashima et al.\textsuperscript{7} reported RBE values of tritium relative to $^{137}$Cs gamma radiation for micronuclei induction and splenic atrophy and the number of bone marrow nucleated cells to be 2.2, 1.5 and 2.4, respectively, for a 2-day exposure to HTO and gamma radiation. For nucleated cell number, RBE of our data is lower than that of Kashima's data. This difference may be due to the difference of the method of measurement of activity and weight of bone marrow, although the present authors can not completely understand their method of dose estimation.

In these reports, the RBE values of tritium relative to $^{60}$Co, $^{226}$Ra or $^{137}$Cs gamma radiation ranged from 1.32 to 2.4. In our experiments, the RBE values of tritium for bone marrow cells were calculated to be 0.91 to 1.51 slightly smaller than those in other reports. On the other hand, Carsten et al.\textsuperscript{5} showed that the late effects of chronic exposure of tritiated water are similar to those of chronic caesium-137 gamma exposure on bone marrow cells in mice. The different RBE value of tritium beta rays may probably be due to the differences in reference radiation, dose rate, and the method used to determine absorbed dose of tritium in tissue, as well as the biological endpoints.
As mentioned by the NCRP\textsuperscript{8}, the RBE of beta radiation from tritium can be changed by a factor of approximately 2 depending upon whether gamma rays or X rays are used for the reference radiation at high doses and dose rates. Furthermore, in his review Bond\textsuperscript{9} notes that the RBE of low energy (50-200kVp) X-rays at low dose and/or low dose rates would be approximately 2.5 or 3 using \textsuperscript{60}Co gamma rays as the reference radiation. Thus, the RBE value of tritium to X-rays may become 1 or near 1, if the RBE value of tritium to a \textsuperscript{60}Co gamma is about 2. Vennart\textsuperscript{10}, quoting several reports, some of which are mentioned above, has suggested that a quality factor (QF) different from unity for tritium beta rays is hardly justified.

Recently, ICRU\textsuperscript{11} notes that, according to the concept of lineal energy, a factor of 2 is an acceptable approximation value of \( Q \) (effective quality factor) for tritium beta rays in radiation protection operations. However, our present data suggest that the RBE of the \( ^{3}H \) beta particle may be slightly greater than 1 but less than 2 for acute effect on bone marrow cells in the mouse.

\section*{ACKNOWLEDGMENT}

This investigation was supported in part by Scientific Research Fund Nos. 59050019 and 60050020 from the Ministry of Education, Japan.

\section*{REFERENCES}