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Original Article

Tissue distribution, excretion and effects on genotoxicity of tritium following oral administration to rats

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ABSTRACT

Tritium is an important nuclide that must be monitored for radiation safety management. In this study, HTO was orally administered to rats at the level of 37 kBq (1 μCi) or 370 kBq (10 μCi) to examine tissue distribution and excretion levels. After sacrifice, wet and dry tissue samples were weighed and analyzed for tissue free-water tritium (TFWT) and organically bound tritium (OBT). The mean tissue concentrations of TFWT (OBT) were 30.9 (17.8) and 4.4 (8.1) Bq/g on days 7 and 13 at the 37 kBq level and 30.8 (64.6) Bq/g on day 17 at the 370 kBq level. To assess the cytogenetic damage due to tritium exposure, a cytokinesis-blocked micronucleus (MN) assay was performed in blood samples from rats exposed to HTO for 14 and 21 days after oral administration. There was no significant difference in the MN frequencies between the control and exposed rats.

1. Introduction

Tritium (^{3}H) is a radioactive isotope of hydrogen. It is a beta emitter with a maximum decay energy of 18.6 keV (average 5.7 keV), and a physical half-life of 12.3 years. External beta radiation does not pose a danger because it cannot penetrate the skin surface, but internal beta-ray exposure can cause damage. Beta radiation occurs widely as a result of the reactions of atmospheric atoms with cosmic rays, in nuclear fission, and in activation reactions. Tritium is mainly generated by ternary fission in nuclear fuel and by neutron reactions with light elements such as boron and lithium in control rods or burnable poison dissolved in the primary water coolant. The amount of tritium generated in a pressurized heavy water reactor (PHWR) by neutron capture reactions of deuterium in heavy water exceeds that in a light water reactor (LWR) by almost a 100 times. In South Korea, there are four PHWR at Wolseong [1–3]. Periodic monitoring of tritium levels is performed for the safety of the residents and the environment livings in the nearby nuclear power plant. However, tritium is still detected in the urine samples from the residents around nuclear power plant, which may cause concern and anxiety to the health of the residents.

Tritium can be absorbed in a variety of forms through occupational and general exposure via inhalation and drinking water, ingestion of food, and through the skin. In animal bodies tritium exists mainly in two chemical forms, tissue free-water tritium (TFWT) and organically bound tritium (OBT) [4,5]. Although most tritium, in the form of TFWT, is circulated and excreted into internal bodies, some tritium, in the form of OBT, combines with organic substances such as lipids, proteins, and nucleic acids in tissues or cells. As the duration of tritium presence in a living organism increases, it is more likely to cause direct negative effects and chromosomal aberrations [6–8].

Micronuclei (MN), small nuclei outside the main nucleus, have been used to indicate chromosomal structural aberrations in various cases of chronic disease conditions and environmental

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toxicity. MN assay is extensively used in the field of ionizing radiation to evaluate the accumulation of cytogenetic damage in an individual following accidental radiation exposure, including exposure to tritium [9,10]. Chemicals, oxidative damage, and radiation are known to affect MN frequency, making MN a significant indicator of the effects of tritium in animal models [11,12]. To confirm whether it is involved in biological effects and genetic damage, the distribution and retention of tritium in various rat tissues have been determined after the administration of low-level tritiated water. Cytogenetic damage due to exposure to tritium was also investigated via MN assay.

2. Materials and methods

2.1. Animals

The animals used were 5-week-old male rats (Sprague-Dawley, Orient Bio Ltd, Korea) weighing about 195 g at the time of exposure. The rats were individually maintained in an environment with water and food freely available at all times, according to the guidelines of the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Committee for the Protection and Use of Animals.

To efficiently investigate the tritium level of urine and feces as an estimate of tritiated water, we made cages with the design of the plastic trays and glass separation cone. The dimensions of cage are 20 cm (length) × 20 cm (width) × 45 cm (height).

2.2. Tritiated water (HTO) experiment

To administer a convenient amount of HTO orally to rats, a calibrated tritiated water standard was used at a concentration of 37 kBq or 370 kBq/0.5 ml. Tritium was administered to rats using a micropipette into the mouth. The daily excretion of HTO in urine was examined for 17 days. Following oral administration of HTO, incorporation of tritium into rat tissues was examined in both wet and dry samples of heart, lung, liver, kidney, colon, stomach, testes, muscle, and blood. All samples, including the blood, were weighed and then frozen in sealed plastic tubes as soon as possible to prevent the loss of tritium and to preserve the sample. The TFWT of the tissue samples was extracted using a freeze-drying vacuum system. The water obtained was mixed with a liquid scintillation counter (LSC) cocktail solution and HTO activity concentrations were determined directly using an LSC. Urine and fecal samples were obtained from the animals every 24 h. Tritium concentrations in the urine sample were analyzed by LSC. The TFWT of each organ and fecal sample was extracted using a freeze-drying vacuum system. The samples were loaded into vacuum flasks and exposed to dry ice traps at a pressure of 0.013 Pa and temperature of −65 °C for 24–36 h. Of the water obtained, 80 μl of the water obtained was mixed with 8 ml of 0.1M NHO3 and 12 ml Ultima Gold XR (Perkin-Elmer, USA). The vials were placed in a Quantulus 1220 LSC and counted for 100 min (with a typical background of 1 cpm) to determine the TFWT activity of the sample. The minimum detectable activity was approximately 0.004 Bq/ml.

2.3. Organically bound tritium (OBT) experiment

Tissue samples remaining after the TFWT analysis were chopped and homogenized and mixed with 10–20 ml of tritium-free water to remove the exchangeable OBT. The samples were then refrozen and subjected to a second round of cryogenic distillation under vacuum. This process was repeated at least twice, until the tritium concentration of the rinse-water resembled the background value. The rinsed samples were then combusted using an oxidizer with pressurized oxygen. The combustion water from these samples was analyzed by LSC to determine OBT concentrations.

2.4. Micronucleus assay

The MN assay was part of a genotoxicity test for the detection of MN in the cytoplasm of interphase cells. For MN assay, cytochalasin B at a concentration of 3 μg/ml was added to the blood samples at h 24 and cells were harvested at h 72 after hypotonic treatment with 0.45% of cold KCl. Cells were fixed in Carnoy's solution and stained with Giemsa. At least 1000 binucleated cells were scored to assess the frequency of cells with MN.

2.5. Statistical analysis

All experimental data are reported as mean and the error bars represent the experimental standard error. Statistical analysis was performed by the non-parametric Student t-test.

2.6. Calculation of average absorbed dose

For total body exposure to radiation for time (t) after administration of HTO, the average absorbed dose was calculated based on used tissue distribution factor (TDF) and defined as follows [13,14]:

Table 1. Concentration of administrated HTO and sacrifice day for sample in rats.

The absorbed dose (D) accumulated in TWFT and OBT in a period from 0 to t days was calculated using the following Formula (1):

$$D(t) = C \int_0^t Q0(t)dt \quad (1)$$

Where C is the conversion factor of 7.87×10^{-8} Gy·g/Bq·day and Q_0 represents the initial body burden of tritium per unit mass Bq/g.

3. Results

3.1. The concentration of tritium in the urine and feces of rats

We first examined the concentration of tritium in the urine and feces of rats that received 37 kBq (1 μCi) or 370 kBq (10 μCi) HTO orally. The daily excretion of HTO in urine was examined until day 17. At the 370 kBq level, the mean tritium concentration of urinary excretions was about 2243.79 ± 75.02 Bq/ml at day 1, and 46.0 ± 7.99 Bq/ml at day 17 (Fig. 1A). A similar excretion pattern

Table 1
Concentration of administrated HTO and sacrifice day for sample in rats.

Experiment number	Activity administered	Day sacrificed	Samples	Organ	Organ
1	10 μCi	17	Urine, Organ	Heart, Lung	Heart, Lung
2	1 μCi	13	Urine, Feces, Organ	Liver, Blood	Liver, Blood
3	1 μCi	7	Organ	Kidney, Colon	Kidney, Colon
4	1 μCi	14	Blood (MN)	Stomach, Testes	Stomach, Testes
5	1 μCi	21	Blood (MN)	Muscle, Bladder	Muscle, Bladder

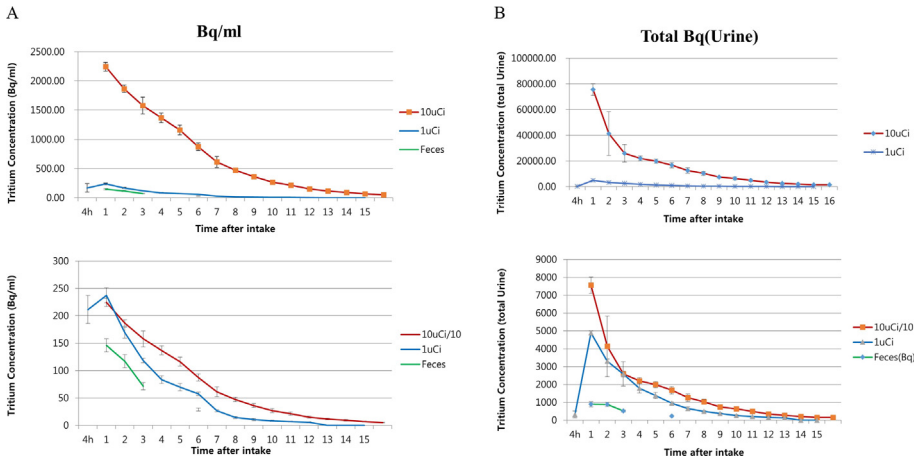


Fig. 1. Excreted tritium concentration of urine after administration of 37 or 370 kBq tritiated water in rats.
(A)Tritium concentration of excretion as Bq ml^{-1} of urine of 37 or 370 kBq (upper panel) and state of 370 kBq divided by 10 (lower panel). Concentration with extracted tritiated water of feces using the freeze-dried feces sample (green line). (B) Tritium concentration of excretion as total activity (Bq) of urine after administration of 37 or 370 kBq (upper panel) and state of 370 kBq divided by 10 (lower panel).

was observed after exposure to the lower level of 37 kBq; the mean concentration was about 237.38 ± 13.92 Bq/ml at day 1 and 5.39 ± 1.12 Bq/ml at day 13, one tenth that of the 370 kBq level. The maximum concentration of 218 Bq/ml was investigated according to the survey of tritium concentration in urine samples of radiation workers from Korea PHWR for 1 day and continuous monitoring is required if the concentration of tritium exceeds 370 Bq/ml in urine samples [15]. When 37 kBq tritium was administered to the animals, it became similar to the maximum tritium concentration in urine excreted by the radiation workers. Although biological metabolism, the biological half-life of tritium and the amount of excreted urine between humans and animals are quite different, at this concentration of tritium, biological effect of an animal model is actually expected to be greater than that of humans. This is because the concentration of trititum in urine relative to body weight is higher in rats that in humans. Therefore, experiments were performed to determine whether tritium can cause genetic damage in animals that secrete >200 Bq/ml into urine.

In Fig. 1B, the value of the total activity (Bq) is that of the activity per unit urine (Bq/ml) multiplied by the total quantity of the urine samples. Approximately 20.43 ± 6.86% of the rate was excreted in urine during the first 24 h and a further 65% was excreted between days 1 and 10, with very low but detectable levels 10 days later in the 370 kBq level. Urinary excretion was high at day 1 and declined slowly after day 2. The concentrated dose of the fecal excretion accounted for 22.29 ± 3.92% of urinary excretion rate at day 1. In previous studies, the distribution of tritium inside the body was rapidly equilibrated throughout the total body water. In humans, tritium concentrations reached equilibrium within 2 h, according to urine samples [15]. The amount of urine in rats was measured at 4 h, when the collection of samples was possible. In case of 37 kBq, the tritium concentration after 4 h was about 211.47 ± 25.51 Bq/ml, indicating that the tritium inside the body had not yet reached concentration equilibrium compared to the concentration of tritium at 24 h in rats.

We measured tritium concentrations from the extracted tritiated water of feces using freeze-dried feces samples. The tritium concentration per unit water (Bq/ml) and total activity of feces was 146.07 ± 11.86 (893.2 ± 142.9), 117.13 ± 12.27 (884.6 ± 122), 71.66 ± 6.6 (513.3 ± 71.1) and 28.95 ± 2.86 (232.3 ± 29.9) Bq/ml at days 1, 2, 3, and 6, indicating that the rate of total Bq in feces was about 22.29 ± 3.92% of the total Bq of urine.

After administration of HTO, the excretion of radioactivity occurred mainly in the urine (65–75%) and to a smaller extent in feces (<15%), indicating that, in rats, urine is the main excretory pathway. Tritium was promptly excreted and the experimental half-time was calculated to be about 3.5 days in rats.

3.2. Organ retention (TFWT and OBT) in rats

Following oral administration of HTO, the incorporation of tritium into rat tissues was examined in both wet and dry samples of the heart, lung, liver, kidney, colon, stomach, testes, muscle, and blood. After sacrifice, these tissue samples were weighed and analyzed for TFWT and OBT. The administered HTO was uniformly distributed throughout the body tissues. The radioactivity of tritium was dependent on the weight and water fractions of the soft tissue. The results showed TFWT and OBT concentrations as shown in Table 2. The mean concentrations of TFWT (OBT) in the tissues of the 37 kBq level rats at days 7 and 13 after dosing were 30.9 ± 5.6 (17.8 ± 4.2) and 4.4 ± 0.6 (8.1 ± 1.0) Bq/g, respectively. The mean concentrations of TFWT and OBT in the tissues of the 370 kBq rats at day 17 after dosing were 30.8 ± 10.4 and 64.6 ± 11.8 Bq/g. The values of TFWT were similar to the urine excretion values observed after exposure to a low level of HTO. No significant differences in TFWT occurred in the weights of the heart, lung, liver, kidney, colon, stomach, testes, muscle, and blood samples. In the case of OBT, the samples from the testes were slightly higher than in the other tissues (Fig. 2A and B). These results suggest that tritium within the body was rapidly and uniformly distributed and excreted immediately with a TFWT consistent with the urine sample. To calculate the value of the half-life, the exponential function of TFWT and OBT was used as shown Fig. 2C. We show that profiles of the mean dose measured in urine, for TFWT and OBT (Bq/g or Bq/ml), suggest that the estimated half-life of TFWT and OBT is about 2.1 ± 0.9 days and 5.9 ± 1.1 days, respectively, in rats. Significantly, the estimated OBT value overtook the TFWT after the administration of tritiated water.

3.3. The pattern of TDF and dose accumulation in low doses of HTO in rats

The pattern of dose accumulation after HTO intake for the urine samples and the pattern estimated based on tritium content in the urine are shown in Fig. 3. The accumulated dose as measured in the urine was about 10-fold higher for the 370 kBq dose than for the 37

Table 2
Measured dose of TFWT and OBT in various organs.

Organ	TFWT (Bq/g) (mean of 4 animals ± SD)									OBT (Bq/g) (mean of 4 animals ± SD)								
	1 μCi						10 μCi			1 μCi						10 μCi		
	Day 7			Day 13			Day 17			Day 7			Day 13			Day 17		
Heart	31.9	±	10.8	3.9	±	0.6	36.0	±	4.2	17.5	±	3.8	9.4	±	1.0	56.4	±	21.8
Lung	32.6	±	5.7	4.2	±	0.5	28.7	±	14.7	18.3	±	5.7	8.8	±	1.1	68.7	±	14.8
Liver	31.2	±	4.4	4.4	±	1	29.3	±	6.1	18.5	±	3.9	6.1	±	0.5	53.7	±	2.4
Blood	31.2	±	6.6	4.2	±	0.9	31.0	±	10.4	13.5	±	4.2	8.4	±	1.5	54.9	±	4.8
Kidney	31.2	±	4.8	4.3	±	0.5	31.5	±	8.4	21.1	±	3.5	8.5	±	1.3	60.7	±	18.0
Colon	31.1	±	5.3	4.4	±	0.4	26.0	±	10.5	12.5	±	4.4	5.1	±	1.9	55.2	±	14.9
Stomach	29.1	±	4.8	4.2	±	0.5	30.2	±	20.7	18.1	±	3.2	6.9	±	1.5	66.9	±	4.8
Testes	31.3	±	4.9	4.6	±	0.6	39.9	±	8.5	22.1	±	3.2	9.1	±	0.0	86.9	±	19.2
Muscle	28.8	±	3.4	5.0	±	0.4				18.6	±	5.5	10.3	±	0.2			
Bladder							25.0	±	9.7							77.4	±	5.8
AVERAGE	30.9	±	5.6	4.4	±	0.6	30.8	±	10.4	17.8	±	4.2	8.1	±	1.0	64.6	±	11.8
							3.08*									6.7		

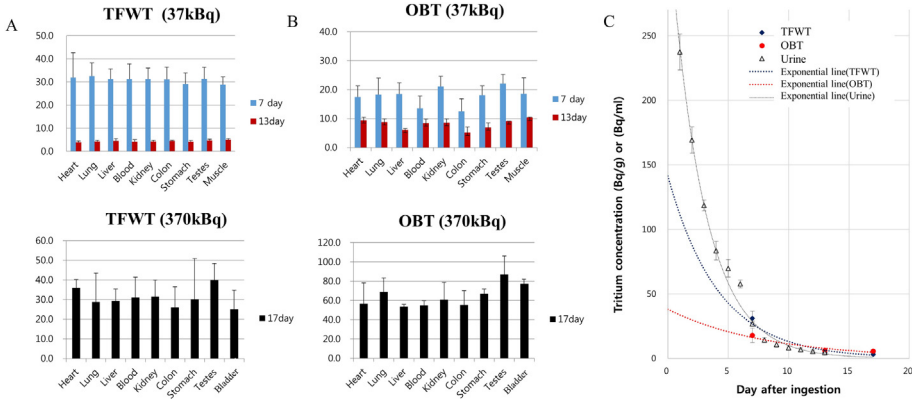


Fig. 2. Variations in the concentrations of TFWT and OBT in several tissues after administration of tritiated water.
(A) TFWT concentration (Bq/g) of tissues after administration of 37 kBq at 7 and 13 days (upper panel) and 370 kBq at 17 days (lower panel). (B) OBT concentration (Bq/g) of tissues of 37 kBq (upper panel) and 370 kBq (lower panel). (C) Profiles of the mean concentration measured in urine, feces, TFWT, and OBT (Bq/g or Bq/ml).

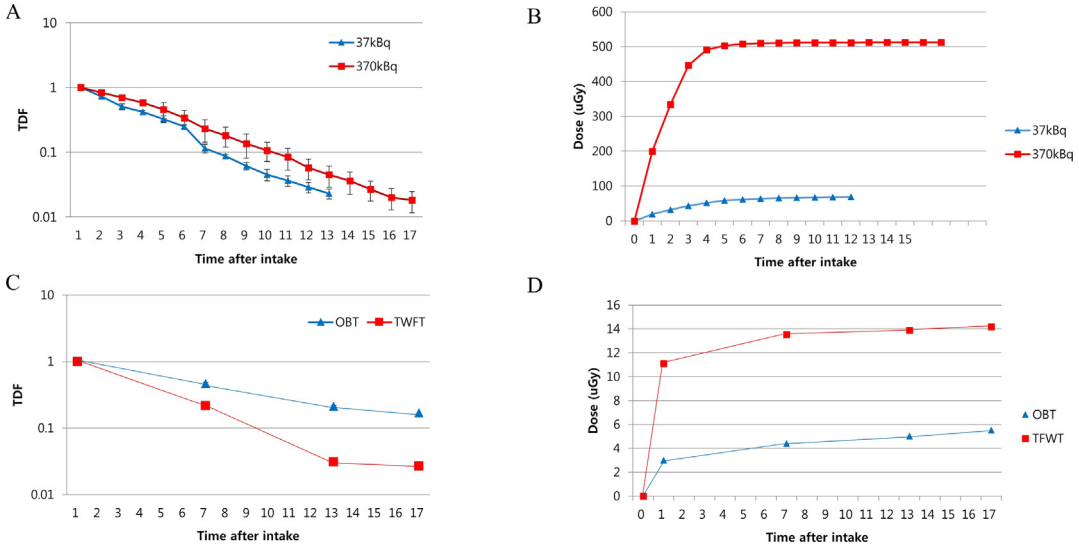


Fig. 3. Values of tritium distribution factor (TDF) and estimated dose accumulation in administration of tritiated water.
(A)TDF value of urine after administration of 37 or 370 kBq tritium in rats. (B) Estimated absorbed dose by urine of 37 or 370 kBq under the assumption that tritium released into the urine remains in the body. (C) TDF values of TWFT and OBT after administration of 37 kBq in rats. (D) Estimated absorbed doses by TFWT and OBT of 37 kBq in rats.

Table 3
Distribution of MN in blood exposed to tritiated water.

37 kBq	Sample	# of binucleated cells	# of MN	MN/1000 binucleated cells
Day 14	Con1	872	16	18.35
	Con2	910	23	25.27
	Con3	898	19	21.16
	Con4	1024	16	15.63
	H1	816	24	29.41
	H2	876	18	20.55
	H3	836	20	23.92
	H4	1146	17	14.83
Day 21	Con5	930	13	13.98
	Con6	1011	21	20.77
	Con7	864	16	18.52
	Con8	1000	18	18.00
	H5	1232	24	19.44
	H6	984	19	19.31
	H7	814	18	22.11
	H8	806	20	24.81

kBq dose after oral administration. The reason that the dose measured at 370 kBq was higher than at the 37 kBq level of tritium is presumed to be due to the amount of water ingested. Strangely, the amount of intake water on the first day was higher about 7 ml in a group of 370 kBq than 37 kBq. Rats ingesting at the 370 kBq level consumed more water for the first 2 days than the 37 kBq rats. For this reason, the TDFu and TDFt values after oral intake at the 37 kBq dose over 17 days had a tendency to be lower than at 370 kBq dose (Fig. 3A). The TDF pattern after oral administration is shown as being consistent with previous studies [13,14]. The patterns of dose accumulation are similar, but there is a significant difference in the value of dose accumulation estimated from the urine concentration between the 37 and 370 kBq. The rapid release of tritium in 37 kBq suggests that the ingestion and excretion of large amounts of water reduces the received radiation dose in the body. The absorbed dose, based on assumption that tritium released into the urine remains in the body and absorbed dose by caused TFWT (or OBT) were shown in Fig. 3B, D. Under this assumption, absorbed doses by urine tritium in the tissues of the 37 and 370 kBq level rats were about 60 and 500 μGy, respectively, for 5 days after oral administration. If not discharged as a urine sample, it may be presented at a dose that bladder or other tissues can be exposed up to 200 μGy for 1 day. The absorbed doses accumulated by TFWT and OBT were about 14 and 5 μGy at day 17 in 37 kBq level rats. As a result, the absorbed dose by TFWT and OBT that can be obtained by oral administration of 37 kBq tritium was estimated to be about 20 μGy.

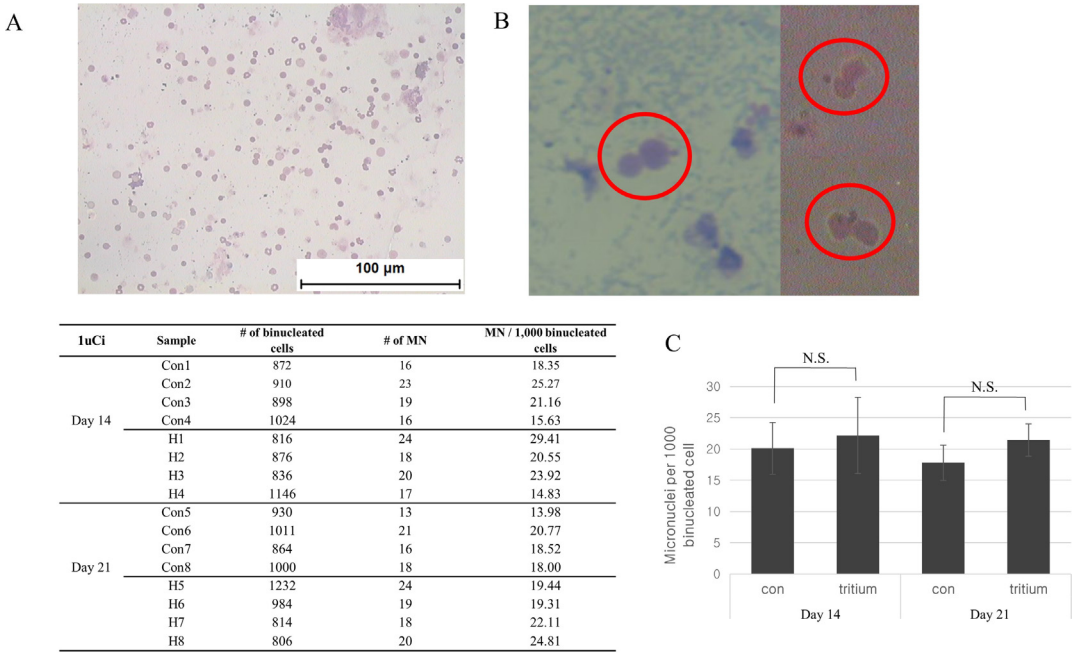
3.4. Micronuclei frequency in low dose of HTO in rats

An MN is formed during the metaphase–anaphase transition of mitosis. The MN assay involves breaking the DNA of lymphocytes in a blood sample with a powerful dose of radiation, then measuring the efficiency of its ability to repair itself. The scoring of MN can be performed relatively easily and on different cell types relevant for biomonitoring of radiosensitivity [17,18]. To assess whether tritiated water influences cytogenetic damage, we examined cytokinesis-blocked MN assay in blood samples from rats exposed to HTO at days 14 and 21 after oral administration.

Table 3 shows that the mean MN frequency counted for rats receiving HTO. Fig. 4A and B shows binucleated cells and binucleated cells with MN. About 1000 binucleated cells were scored from each sample. Frequencies of MN were not found to be significantly different in the blood samples. Background or spontaneous levels of MN frequency were low (13–24 MN per 1000 cells). Data were analyzed to investigate the effect of low-dose tritium exposure. When 16 subjects were grouped by administration of tritium or normal water from the same environmental conditions, the mean frequency showed no significant differences in the MN number estimated from the blood samples (Fig. 4C).

4. Discussion

The objective of this study was to determine the effect of tritium



1uCi	Sample	# of binucleated cells	# of MN	MN / 1,000 binucleated cells
Day 14	Con1	872	16	18.35
	Con2	910	23	25.27
	Con3	898	19	21.16
	Con4	1024	16	15.63
	H1	816	24	29.41
	H2	876	18	20.55
	H3	836	20	23.92
	H4	1146	17	14.83
Day 21	Con5	930	13	13.98
	Con6	1011	21	20.77
	Con7	864	16	18.52
	Con8	1000	18	18.00
	H5	1232	24	19.44
	H6	984	19	19.31
	H7	814	18	22.11
	H8	806	20	24.81

Fig. 4. Binucleated cell with MN and frequency of MN in rat blood.
Binucleated cell with micronuclei in rat blood using a microscope at 10x(A) and 40x(B). (C) Schematic representation of the micronuclei frequency after administration of 37 kBq at 14 and 21 days. No significant difference was evident between these two groups. N.S.: Not significant.

on the distribution of TFWT and OBT in a variety of tissues, as well as the possibility of genetic damage on based on experimental data with low levels of tritium. To confirm the biological effect of HTO, the retention and excretion of tritium in rats was measured after administration of doses of either 37 kBq or 370 kBq level, obtaining data for TFWT and OBT retention. In this experiment, administered tritium was significantly lower than that in the other experiments. Moreover, tritium was not administered several times. The reason is that radiation workers are contaminated with much lower amounts of tritium than we think, and they are not continuously contaminated through the life cycle. Therefore, tritium was singly injected into the rats on the basis of the highest tritium concentration of the urine sample of the radiation workers. Nevertheless, the amount of tritium administered is expected to be considerably high when considering the weight of the individual.

Tritium activity in animal models equilibrates quickly with the internal metabolism. The radiological significance of OBT is greater than that of HTO because of its longer residence time in rat models [6,7,19]. In this study, when large amounts of water were ingested, tritium was released quickly, based on the measured levels of tritium in the urine. The mean concentration of tritium in tissues and urinary excretions indicates that 37 kBq of concentration is quantitatively different from the 370 kBq level, suggesting that it can be quantified as a coefficient (function) at low concentrations of tritium.

The relative contribution of each tissue to the overall effect of exposure to ionizing radiation differs. Consequently, the ICRP recommended and revised weighting factors for different organs and tissues (ICRP, 2007). The testes are sensitive to radiation and their weighting factor was changed from 0.2 in ICRP 1990 to 0.08 in ICRP 2007. Despite the lowered weighting factor for testes, it is still an important organ for considering genetic effects. In this study, the dose of tritium retained in tissues was estimated from the TFWT and OBT of the various tissues; these were not significantly different, although the level of OBT in the testes was slightly higher than in the other tissues.

The tritium concentration measured in the retention and excretion after administration of HTO is consistent with the reported animal models, which suggest tritiated waters are equilibrated and excreted quickly in rat tissues. The faster loss of TFWT and OBT components in rats than in humans is consistent with reported research [19,20]. In this experiment, the estimated half-life of TFWT and OBT was about 2.1 ± 0.9 days and 5.9 ± 1.1 days, while according to the ICRP (1993), for an adult man the half-life is about 10 days and 40 days, respectively, which suggests that the turnover rate of TFWT and OBT is about 5–6 times faster in rats than in humans.

For an accurate assessment of the risk caused by OBT intake via HTO, the localization of the OBT to the target molecule exposed to radiation damage should be considered. Localization of tritium to protein molecules in animal cells is not critical for dose modification. However, in nucleus molecules, localization can result in significant effects in terms of microdose damage. The importance of TFWT and OBT dose contributions should be emphasized for human protection. The TFWT and OBT of blood were considered not to differ significantly, but the MN assay of the blood samples enabled us to measure the overall effect of tritium after oral administration of HTO.

Scanning of MN in cytokinesis-blocked binucleated cells is a relatively easy, accurate, and fast procedure. It is therefore regarded as a sensitive biological marker capable of evaluating the cellular response to irradiation [11,12]. In this study, we confirmed that frequencies of MN were not found to be significantly different in blood samples using MN assay after administrated HTO, which suggests that low-level tritiated water (37 kBq) did not affect induction of MN between the control group and the group exposed to HTO for 14 and 21 days after oral administration in rat blood samples. Although a low level of HTO is thought to be a non-effector in animal models, studies on the specific effects involved in OBT are still lacking. To assess the molecular change or damage of HTO on other tissue samples, specific estimation of absorbed dose on tissues as well as additional genetic and biological approaches, such as γ-H2AX and DNA microarray should be performed.

5. Conclusion

In these study, we measured the distribution and excretion concentration of tritium from urine, feces and tissues in Sprague-Dawley rats following a single administration of HTO. The key results are followings:

1) Tritium was rapidly and uniformly distributed and incorporated into the whole body, despite slight differences in the concentration of tritium in each tissue depending on the water content. Tissue distribution factor (TDF) was used to calculate the average absorbed dose.
2) The concentrations of TFWT and OBT were measured in a variety of tissues. Based on these results, biological half-life of TFWT and OBT in tissues was about 2.1 ± 0.9 days and 5.9 ± 1.1 days in this experiment.
3) There was no significant difference in the micronuclei frequencies between the controls and rats exposed to low level of HTO after oral administration, suggesting that low-level tritiated water (37 kBq) did not affect the induction of micronucleus to assess biological damage.

Conflict of interest

All authors have no conflicts of interest to declare.

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