CHANGES IN BODY AND ORGAN WEIGHTS, HEMATOLOGICAL PARAMETERS, AND FREQUENCY OF MICRONUCLEI IN THE PERIPHERAL BLOOD ERYTHROCYTES OF ICR MICE EXPOSED TO LOW-DOSE-RATE γ-RADIATION

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We exposed ICR mice to low-dose (0.2 Gy) and low-dose-rate (0.7 mGy/h) γ-radiation (^{137}Cs) in the Low-dose-rate Irradiation Facility at the Radiation Health Research Institute to evaluate systemic effects of low-dose radiation. We compared the body and organ weights, number of blood cells (white and red blood cells and platelets), levels of biochemical markers in serum, and frequency of micronuclei in polychromatic erythrocytes between low-dose irradiated and non-irradiated control mice. The ICR mice irradiated with total doses of 0.2 and 2 Gy showed no changes in body and organ weights, number of blood cells (white and red blood cells), or frequency of micronuclei in the polychromatic erythrocytes of peripheral blood. However, the number of platelets ($P = 0.002$) and the liver weight ($P < 0.01$) were significantly increased in mice exposed to 0.2 and 2 Gy, respectively. These results suggest that a low-dose-rate of 0.7 mGy/h does not induce systemic damage. This dose promotes hematopoiesis in the bone marrow microenvironment and the proliferation of liver cells. In the future, the molecular biological effects of lower doses and dose rates need to be evaluated.

Keywords : Radiation, Low-dose-rate, Mice, Organs, Blood, Micronuclei

1. INTRODUCTION

The harmful effects of high-dose radiation on living organisms, including human beings, have become well known since the release of epidemiological data related to the atomic bomb in Japan and the Chernobyl nuclear accident [1]. Generally, experimental animals and cell lines from animals and humans are used to evaluate the biological effects of radiation [2]. Since genetic modification takes place in these cells [3], it is not easy to detect the effects of low-dose and low-dose radiation in individual entity. In other words, the effect of the radiation should be investigated using experimental animals because the physical responses to radiation represented synthetically through responses in cell including DNA, tissue, and organs.

The systemic effect of radiation increases in proportion to the amount of dose and dose rate [4]. However, it has been reported that low-dose radiation induces the repair of damaged DNA [5], reduces apoptosis [6,7], stimulates the immune system [8], and prevents diabetes [9,10] and rheumatoid arthritis [11]. Since the concept and standards of low-dose and low-dose-rate radiation have yet to be established, studies using these types of radiation have not been systematically conducted. A number of recent studies have followed the concept and standards for low-dose ($\leq$ 0.2 Gy) and low-dose-rate ($\leq$ 6 mGy/h) radiation recommended by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) [12]; however, the data from these studies are not sufficient to explain the systemic responses of low-dose and low-dose-rate radiation. In particular, the relationships between harmful systemic effects and accumulated dose, which is derived by continuous

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low-dose-rate radiation exposure, have been little investigated. In this study, we measured body and organ weights, number of blood cells, levels of biochemical markers in blood, and frequency of micronuclei in polychromatic erythrocytes of peripheral blood in order to evaluate the systemic effects of low-dose (≤ 0.2 Gy) low-dose-rate (0.7 mGy/h) radiation in ICR mice.

2. MATERIALS AND METHODS

2.1 Animals

We purchased 6-week-old male ICR mice from the Shizuoka Laboratory (Shizuoka, Japan). These animals were raised in a specific-pathogen-free facility maintained at a temperature of 22 ± 2ºC and 50% ± 10% relative humidity, with a 12 h:12 h light:dark cycle. After an initial 1-week adaptation period, the mice were accommodated in polycarbonate cages (5 mice per cage). Gamma-ray sterilized pellets (Samyang, Korea) and autoclaved water were provided *ad libitum*. All experiments were conducted in accordance with the guidelines for animal experiments of the Radiation Health Research Institute (RHRI), Korea Hydro & Nuclear Power Co. Ltd.

2.2 Irradiation

The long-term Low-dose-rate Irradiation Facility at the RHRI was used to administer low-dose-rate (0.7 mGy/h) radiation. Mice were grouped into 2 dose groups (0.2 and 2 Gy), each of which comprised 5 mice. Control mice for both treatment groups were placed on shelves in the same facility, shielded from the γ-radiation. The dosage received by the mice was determined by an intraperitoneally implanted photoluminescence glass dosimeter (GD-351; Asahi Techno Glass, Tokyo) [13], and the observed dose was calculated as 75% of the ambient dose. The mice were irradiated with low-dose-rate γ-rays for 24 h per day (16.8 mGy/d). Irradiation was stopped for 2 h per week for the purposes of animal care.

2.3 Body and Organ Weights

In order to monitor any changes at the whole-body level, we measured the weights of mice immediately following irradiation. Body and organ (thymus, heart, liver, spleen, lung, and kidney) weights were measured using automatic scales (±0.0001 g, AE240; Mettler, Switzerland). In this study, organ mass relative to body mass was calculated as follow: organ mass (g)/body mass (g).

2.4 Measurement of White and Red Blood Cells and Platelets

Following irradiation, blood was collected from the heart under ether anesthesia. Whole blood was collected using an ethylenediaminetetraacetic acid (EDTA) containing tube (SARSTEDT, Germany), and the number of white blood cells, red blood cells, and platelets were counted using an automatic cell counter (SEAC, Italy).

2.5 Measurement of Biochemical Markers in Blood

Following irradiation, whole blood was collected using a heparin containing tube (SARSTEDT, Germany). The levels of serum biochemical markers (albumin, alkaline phosphatase, alanine aminotransferase, amylase, bilirubin, urea nitrogen, calcium, creatinine, glucose, sodium, protein, and globulin) were evaluated using an animal biochemical marker analyzer (VETSCAN, USA).

2.6 Micronuclei Analysis

We performed the analysis of micronuclei to determine chromosome damage as described previously [14]. Briefly, acridine orange (Merck, Germany) was dissolved in distilled water (1 mg/ml), and 10 μl of this solution was placed on pre-heated (~70 ºC) clean glass microscope slides. Approximately 3 μl of peripheral blood was obtained from the tail vein and smeared onto a glass slide. The analysis of micronuclei in polychromatic erythrocytes was performed under a fluorescence microscope (NIKON E-600, B-2A), and at least 1,000 polychromatic erythrocytes in the peripheral blood were scored per mouse for each data point.

2.7 Statistical Analysis

Comparisons of the body and organ weights, number of blood cells, biochemical markers, and frequency of micronuclei in the peripheral blood erythrocytes in irradiated and non-irradiated mice were performed using Student's *t*-test. All statistical analyses were performed using the SAS 8.0 statistical package (SAS Institute, USA).

3. RESULTS

The comparison of the systemic changes in the irradiated and non-irradiated mouse groups revealed that the number of platelets (P = 0.002, Table 3) was greater in the 0.2-Gy-irradiated mice and that liver weight (P = 0.0019, Table 2) and sodium levels in the blood (P = 0.004, Table 4) were greater in the 2 Gy irradiated mice than the non-irradiated control mice. The number of micronuclei in the polychromatic erythrocytes remained unchanged in both the 0.2 and 2 Gy irradiated mice (Table 5).

4. DISCUSSION

In this study, we evaluated the systemic response of

ICR mice to low-dose-rate (0.7 mGy/h) radiation in accordance with the International Commission on Radiological Protection (ICRP) recommendations for low-dose (≤ 0.2 Gy) and low-dose-rate (≤ 6 mGy/h) radiation [12]. Firstly, we measured body and organ weights after low-dose irradiation with 0.2 and 2 Gy. It has been assumed that if systemic damage occurs, it would be expressed as weight loss because it would take 11.9 and 119 days to reach 0.2 and 2 Gy, respectively. In addition, it has been believed that weight loss would gradually bring about cellular-leveled changes. Since numerical errors can occur in the measurement of organ weight, depending on blood volume, water content in organs was sampled by the same researcher. We observed no changes in the body weight of the irradiated mice (Table 1). This finding is similar to that observed in the bank voles sampled from the site of the Chernobyl nuclear accident (0.07 Gy/y) [15]. However, the body weight of shrews from the same site increased. These results demonstrate an interspecific difference in sensitivity to radiation among small wild animals. Interestingly, ICR mice irradiated with a total dose of 2 Gy showed an increase in liver weight (P = 0.0019, Table 2), which is similar to the weight increases observed in the liver of bank voles and in the liver, kidney, and spleen of shrews. They also reported that the body weight and organ weight of CBAxC57BL/6 F1 mice decreased after irradiation with a total dose of 0.07 Gy. However, changes in the levels of serum biochemical markers, which indicate systemic damage, have not been examined. In this study, the ICR mice irradiated with a total dose of 2 Gy showed an increase in liver weight and the serum levels of alkaline phosphatase, alanine aminotransferase, and bilirubin were stable. Therefore, the mice exposed to low-dose-rate irradiation of 2 Gy did not appear to have undergone any pathological change in body. The increase in liver weight is similar to an increase in the proliferation of human fibroblast cells after irradiating with a total dose of 0.05 Gy [16,17] and the increase in the number of organ cells in 0.07 Gy/y irradiated bank voles [15]. Therefore, our data suggests that 0.7 mGy/h has an effect on liver cell proliferation.

Table 1. Body Weights of ICR Mice after Low-dose-rate Irradiation.

Dose (Gy)	Body weights (g)	
	Control	Irradiation
0.2	40.7±1.4	41.7±2.5
2	53.6±8.7	54.5±3.9

Body weight (g) was measured at 11.9 days (0.2 Gy) and 119 days (2 Gy) after low-dose-rate (0.7 mGy/h) irradiation.

Table 2. Ratio of Organ to Body Weights after Low-dose-rate Irradiation.

Organs	0.2 Gy		2 Gy	
	Control	Irradiation	Control	Irradiation
Thymus	0.001±0.0003[#]	0.001±0.0003	0.0006±0.0002	0.0005±0.0002
Heart	0.005±0.0004	0.005±0.0007	0.004±0.0008	0.005±0.0006
Liver	0.054±0.003	0.050±0.002	0.042±0.002	0.049±0.002[*]
Spleen	0.003±0.0003	0.003±0.0004	0.003±0.001	0.002±0.0002
Lung	0.006±0.0003	0.006±0.0007	0.005±0.001	0.005±0.0006
Kidney	0.008±0.0009	0.008±0.0007	0.006±0.001	0.006±0.001

Organ weights were measured at 11.9 days (0.2 Gy) and 119 days (2 Gy) after low-dose-rate (0.7 mGy/h) irradiation.
[#] Ratio of organ weigh = organ weight(g)/body weight(g).
[*] P = 0.0019. Mean ± SD (n = 5/group).

Table 3. Hematology in Peripheral Blood of Low-dose-irradiated ICR Mice.

Hematology	0.2 Gy		2 Gy	
	Control	Irradiation	Control	Irradiation
White blood cell (K/Ul)	4.9±1.9	5.4±1.7	4.5±1.7	3.4±0.8
Red blood cell (M/Ul)	8.7±0.3	9.4±1.4	7.9±0.1	7.9±0.2
Platelet (K/Ul)	966±199	1,386±74[*]	1,400±227	1,215±125

White blood cell, red blood cell and platelets were measured at 11.9 days (0.2 Gy) and 119 days (2 Gy) after low-dose-rate (0.7 mGy/h) irradiation. [*] P = 0.002. Mean ± SD (n = 5/group).

Table 4. Biochemical Markers in Serum of Low-dose-rate Irradiated ICR Mice.

Chemical markers	0.2 Gy		2 Gy	
	Control	Irradiation	Control	Irradiation
Albumin (g/dl)	3.5±0.1	3.4±0.1	3.4±1.0	3.9±0.1
Alkaline phosphatase (IU/L)	49±24	65±23	27±8	23±17
Alanine aminotransferase (IU/L)	24±6	23±7	27±5.3	33±5.6
Amylase (IU/L)	1,007±129	863±91	1,046±216	1,314±160
Bilirubin (mg/dl)	0.3±0.1	0.3±0.0	0.2±0.1	0.2±0.0
Urea nitrogen (mg/dl)	20±2.0	21±4	25±3.3	30±2.7
Calcium (mg/dl)	10±0.4	9.8±0.3	9.8±0.1	9.9±0.3
Creatinine (mg/dl)	0.2±0.0	0.2±0.04	0.2±0.0	0.2±0.0
Glucose (mg/dl)	117±4.3	128±18	153±36	115±21
Sodium (mmol/L)	153±3.4	152±3.1	149±2.2	153±2.2*
Protein (g/dl)	5.4±0.2	5.2±0.1	5.8±0.4	5.8±0.2
Globulin (g/dl)	1.9±0.2	1.9±0.1	1.8±0.2	1.9±0.2

Chemical markers in serum were measured at 11.9 days (0.2 Gy) and 119 days (2 Gy) after low-dose-rate (0.7 mGy/h) irradiation (^{137}Cs).
* P = 0.004. Mean ± SD (n = 5/group).

Table 5. Micronucleus in Polychromatic Erythrocytes of Peripheral Blood of 2 Gy γ-irradiated Mice.

Dose (Gy)	Control	Irradiation
0.2	0.0003±0.0005	0.0002±0.0004
2	0.0002±0.0004	0.0002±0.0003

The frequency of micronuclei in polychromatic erythrocytes was calculated after low-dose-rate (0.7 mGy/h) γ-irradiation (^{137}Cs).

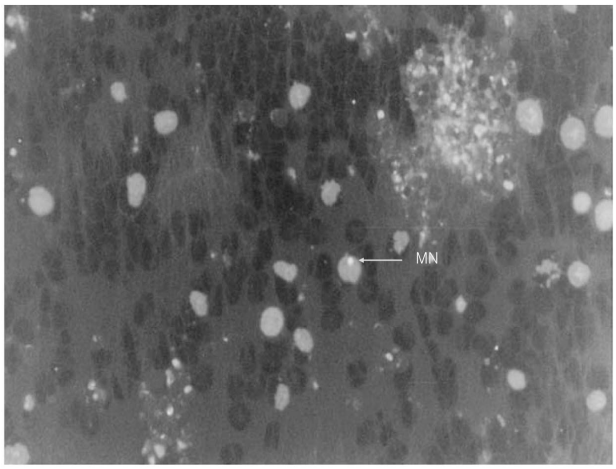

Fig. 1. Acridine orange stained yellow-green colored micronucleus (MN, arrow) in polychromatic erythrocytes of peripheral blood of 2 Gy γ-irradiated mice.

Secondly, we measured white and red blood cells and platelets in blood after low-dose-radiation with 0.2 and 2 Gy as an indicator of systemic damage. It has been suggested that if the bone marrow or organ cells are damaged, then in addition to changes in body and organ weights, a change in the number of blood cells should also be observed. However, no difference in the number of blood cells was observed in the 0.2 Gy irradiated mice with the exception of platelets (P = 0.002, Table 3) in this study. Moreover, the frequency of micronuclei in the ICR mice irradiated with a total dose of 0.2 Gy was not significantly different. This result indicates that the bone marrow was not affected by low-dose and low-dose-rate radiation. Therefore, the myeloid stem cells related to platelets in the bone marrow are probably stimulated by low-dose-rate radiation. These data are consistent with those reported by Wu et al [4] and Goodman et al [18]. They reported increases in the number of bone marrow cells and platelets in mice after irradiation with a total dose of 0.2 Gy, even though they irradiated mice at a high dose rate.

Finally, we measured the levels of biochemical markers in blood after low-dose-rate irradiation with 0.2 and 2 Gy as another marker of systemic damage. It is believed that systemic damage induced by radiation will be reflected by changes in the levels of serum biochemical markers. However, we did not observe any remarkable change in the blood levels of biochemical markers in the mice irradiated with 2 Gy, with the exception of the amounts of sodium. It is well known that sodium concentrations in blood are associated with kidney dysfunction (urea, nitrogen, and creatinine), dehydration, and malnutrition (albumin, glucose, and protein).

Therefore, it is difficult to conclude that the increase in sodium levels was caused by radiation because the levels of related biochemical markers in blood were stable.

5. CONCLUSION

Taken together, our results indicate that irradiation of ICR mice with cumulative doses of 0.2 and 2 Gy, at a low dose rate (0.7 mGy/h), has little or no effect on body and organ weights or on the bone marrow. However, the number of platelets in the blood of mice irradiated with 0.2 Gy and the number of liver cells in those irradiated with 2 Gy were increased. These data suggest that low-dose-rate radiation has no harmful effects on mice. Nevertheless, platelet related myeloid stem cells in the bone marrow were stimulated and liver cells proliferated dose dependently following low-dose-rate radiation. Therefore, further study needs to be conducted on the molecular biological reactions of organ cells in animals subjected to low-dose and low-dose-rate irradiation.

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