DOSE AND DOSE RATE EFFECTS OF IRRADIATION ON BLOOD COUNT AND CYTOKINE LEVEL IN BALB/c MICE

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The biological effects of radiation are dependent on the dose rate and dose of radiation. In this study, effects of dose and dose rate using whole body radiation on plasma cytokines and blood count from male BALB/c mice were evaluated. We examined the blood and cytokine changes in mice exposed to a low (3.49 mGy h^{-1}) and high (2.6 Gy min^{-1}) dose rate of radiation at a total dose of 0.5 and 2 Gy, respectively. Blood from mice exposed to radiation were evaluated using cytokine assays and complete blood count. Peripheral lymphocytes and neutrophils decreased in a dose dependent manner following high dose rate radiation. The peripheral lymphocytes population remained unchanged following low dose rate radiation; however, the neutrophils population increased after radiation. The sera from these mice exhibited elevated levels of flt3 ligand and granulocyte-colony-stimulating factor (G-CSF), after high/low dose rate radiation. These results suggest that low-dose-rate radiation does not induce blood damage, which was unlike high-dose-rate radiation treatment; low-dose-rate radiation exposure activated the hematopoiesis through the increase of flt3 ligand and G-CSF.

Keywords: Radiation, Dose rate, Blood, Cytokine assay, G-CSF, flt3 ligand

1. INTRODUCTION

The possible role of exposure to radiation as a risk factor for human health has been of increasing public concern in the series of explosions at earthquake damaged nuclear reactors on the Japan. Current events throughout the world underscore the growing threat of different forms of accidental exposure to radiation including nuclear accidents, atomic weapons use and testing, and the side effects of cancer therapy. A large range of dose rates of ionizing radiations could be encountered in accidental radiation situations. Nevertheless, most of the studies related to radiation effects have only examined a high dose rate.

The systemic effect of radiation increases in proportion to the dose amount. Cumulative evidence has revealed that radiation is related to immunosuppression in humans and animals [1]. However, it was recently reported that some low dose radiation has the potential to induce immunopotentiation [2,3]. Since effects for low dose and low dose rate radiation have yet to be established, studies using these types of radiation have not been systematically conducted. Previous studies have demonstrated that cytokine production and secretion increases in response to radiation; however, few studies have examined these effects at a low dose rate of radiation. In this study, we investigated the blood count and the plasma cytokine levels of mice exposed to a high or low dose rate of radiation.

2. MATERIALS AND METHODS

Male 7-week-old BALB/c mice (Central Lab. Animal, Seoul, Korea) were used after one week of quarantine and acclimatization. The mice were divided randomly into six group: (1) Sham low dose rate radiation exposure, (2) 0.5 Gy low dose rate (3.49 mGy h^{-1})

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radiation exposure, (3) 2 Gy low dose rate (3.49 mGy h^{-1}) radiation exposure, (4) Sham high dose rate radiation exposure, (5) 0.5 Gy high dose rate (2.6 Gy min^{-1}) radiation exposure, and (6) 2 Gy high dose rate (2.6 Gy min^{-1}) radiation exposure, and each group was composed of seven mice. The absorbed dose was measured using a glass dosimeter that was embedded in the mouse abdomen by a surgical operation. Small-scale glass chips (GD-301; 8.5 mm in length, 1.5 mm in diameter) were specially designed for use in this experiment. In which the chips were embedded in the mouse peritoneal cavity by a simple surgical operation under anesthesia using tiletamine/zolazepam (Zoletil 50®; Virbac Korea, Seoul, Korea). Before performing the readout procedures after exposure, the chips were removed from the mice, washed with 70% ethanol and sonicated in distilled water. Mice were exposed to a low dose rate of gamma-ray radiation in a specific pathogen free conditioned irradiation room equipped with a ^{137}Cs source (185 GBq). Mice were placed in a cage on shelves located 2 m from the source representing 0.5 and 2 Gy (3.49 mGy h^{-1}) exposure and reared for 1 day. Radiation exposure was continued for almost 24 hours a day except for 2 hours a week during which the room was cleaned, bedding changed and food and water refreshed. Sham control mice for both treatment groups were placed on shelves in the same facility and were shielded from the radiation. High dose rate of gamma-ray radiation was carried out in ventilated Plexiglas containers and the mice received 0.5 or 2 Gy total body radiation using ^{137}Cs, 81.4 TBq (2200 Ci) gamma-rays (Eckert & Ziegler, Berlin, Germany) at a dose rate of 2.6 Gy min^{-1}. Sham exposure consisted of placing the mice in identical closed Plexiglas tube for the same period in the absence of irradiation. The animals were maintained in a room at 23±2ºC, with a relative humidity of 50±5%, artificial lighting from 08:00–20:00 and 13–18 air changes per hour. Animals were maintained at an animal care facility, and food and water were supplied *ad libitum*. All animal experiments were conducted following a protocol approved by the Institutional Animal Care and Use Committee of the DIRAMS.

The mice were sacrificed 1 day after radiation. Blood samples were collected from the intracardiac puncture and collected in sample tubes containing EDTA, and peripheral white blood cells (WBC), including neutrophil, lymphocyte, eosinophil, and basophil and platelet counts were monitored. The number of blood cells was automatically counted using a HEMAVET (DrewScientific Inc., UK). The remaining blood was centrifuged at 4500 rpm for 20 min, and plasma was collected and preserved at –80°C. Assays were performed according to the manufacturer's instructions, and cytokine concentrations were calculated using the sofmax program and standard curves (3.2~10,000 pg/ml). The production of interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17, tumor necrosis factor (TNF-α), granulocyte-colony-stimulating factor (G-CSF), interferon (IFN)-g and vascular endothelial growth factor (VEGF) were measured using Lincoplex mouse cytokine kits (Linco, Research, Inc). Plasma flt3 ligand (FLT3) concentrations were measured by ELISA according to the manufacturer's recommendation (R&D Systems, London, UK). The data are reported as the means ± SEM. The data were analyzed using one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls *post hoc* test for multiple comparisons. In all cases, a P value < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

Previous study showed that the number of leukocyte and lymphocyte were dose-dependently decreased after high-dose-rate (21 Gy h^{-1}) irradiation exposure at dose levels of 0.5 Gy or more in mice [4]. However, the low-dose-rate (3.49 mGy h^{-1}) irradiation exposure did not cause hematopoietic injury in mice at dose levels of 2 Gy or less [5]. In this study, we selected the irradiation dose of 0.5 and 2 Gy for comparison with high/low dose rate irradiation.

As shown Fig. 1, the white blood cells including lymphocytes and neutrophils decreased after high dose rate radiation exposure, and this decrease was dose dependent. On the other hand, low dose rate radiation did not exert any substantial changes in blood cells, while only neutrophils were increased in a dose dependent manner.

The dose rate is one of the principal factors that determines the biological consequences of a given radiation dose. In general, as the dose rate is lowered, the biological effect of a given dose is reduced [6]. Radiation reduces the number of stem and progenitor cells, which in turn reduces the turnover of circulating cells [7]. As a result, leucopenia may appear, with an increased risk of opportunistic infections. These effects are dependent on both the radiation dose and the heterogeneity of the irradiation [6]. Nevertheless, if variations in the number of lymphocytes and neutrophils are strictly dependent on the radiation dose, then variations in the dose rate may not affect the observed correlation between blood count and radiation dose. In this study, the mice exposed to a low dose rate of radiation

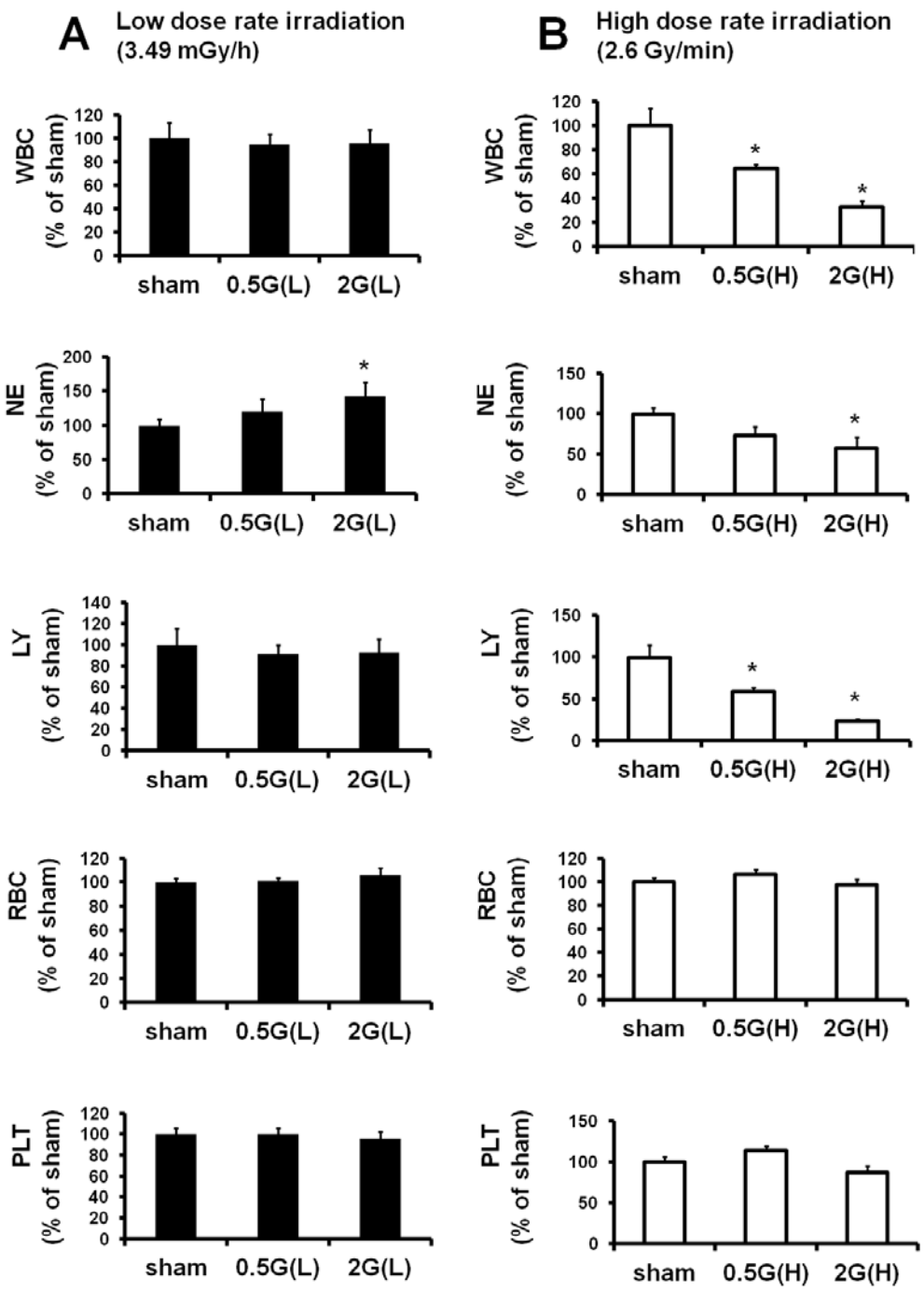

Fig. 1. Effects of low-dose-rate and high-dose-rate of irradiation on the number of white blood cell (WBC), Neutrophil (NE), Lymphocyte (LY), Red blood cell (RBC), and Platelet (PLT) in mice peripheral blood. A low-dose-rate of radiation increased in the number of NE in a dose dependent manner; however, there were no changes in the WBC, LY, RBC and PLT populations (A). The high-dose-rate of radiation decreased the number of WBC, NE and LY in a dose dependent manner (B). Data are presented as the mean ± standard error of the mean (n=7). *P < 0.05 as compared to sham.

showed an increase in the number of neutrophils. The increase in the number of neutrophils was similar to the proliferation of human fibroblast cells after irradiating with a total dose of 0.05 Gy [8] and the increase in the number of immune cells after 0.07 Gy year^{-1} radiation [9].

As shown in Fig. 2, significantly increased levels of FLT3 were observed in the plasma of all irradiated mice and these changes were dose dependent. G-CSF levels also increased in a dose dependent manner, with statistically significant differences observed in the high/low dose rate 2 Gy radiation group (Fig. 2). The change was prominent for the 2 Gy high dose rate irradiation group. Other cytokines (IL-2, IL-4, IL-5, IFN-g,

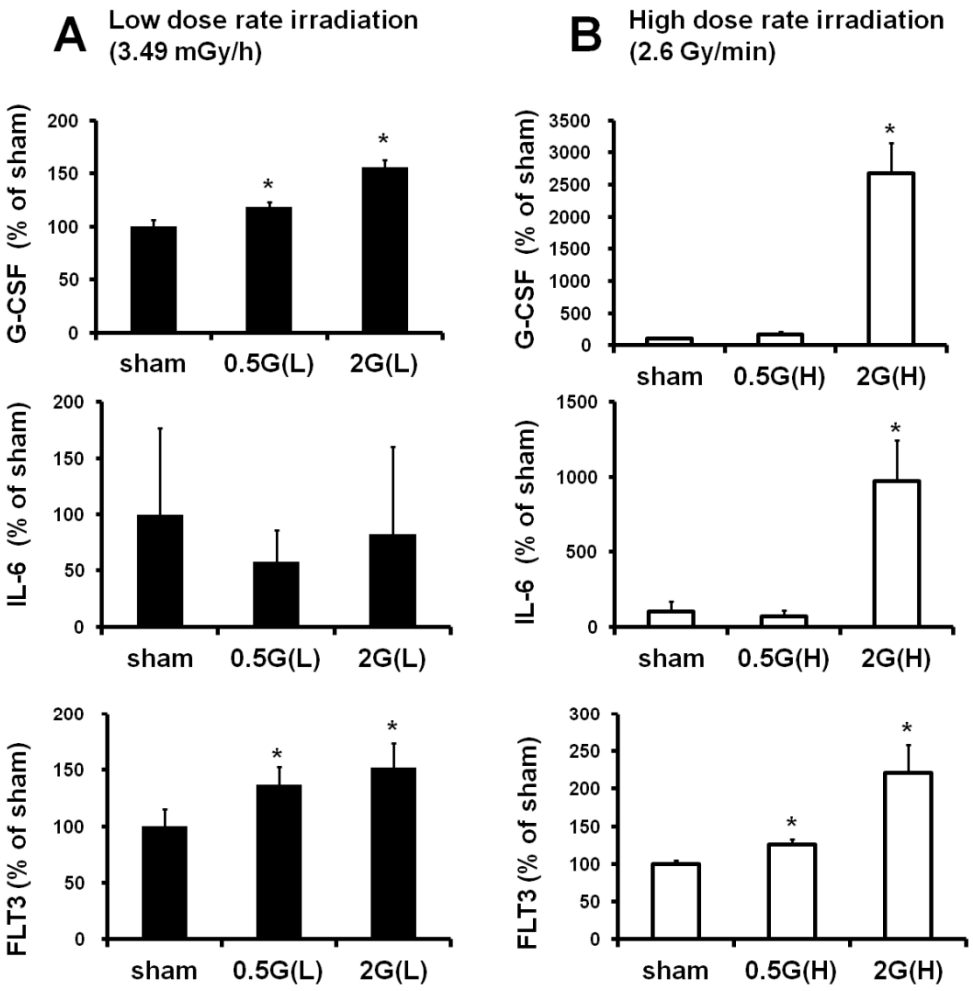

Fig. 2. Effects of the low-dose-rate (A) and high-dose-rate (B) of irradiation on the cytokine levels of G-CSF, IL-6 and FLT3 in peripheral blood from mice. G-CSF and FLT3 levels dose-dependently increased at the low-dose-rate of radiation (A). IL-6 levels were higher at the high-dose-rate of radiation compared to the low-dose-rate of radiation (B). The relative levels of G-CSF, IL-6 and FLT3 in peripheral blood were presented as the mean ± standard error of the mean (n=7). *P < 0.05 as compared to sham.

VEGF and TNF-α) were near control levels in all radiation exposure groups (data not shown). However, IL-6 was elevated in the high dose rate 2 Gy radiation group by about tenfold over sham controls; however, no differences were observed for the other groups.

Radiation was also followed by the development of increased G-CSF and FLT3 in the peripheral blood plasma. Stimulation of G-CSF and FLT3 production demonstrates that differentiation, recruitment and activation of myeloid cells occurred in response to irradiation treatment [10, 11]. G-CSF is a pleiotropic cytokine that influences differentiation, proliferation and activation of the neutrophilic granulocyte lineage. FLT3 can also mobilize hematopoietic primitive and committed progenitor cells into the peripheral blood of mice and those cells mobilized by FLT3 can be used for peripheral blood stem cell transplantation [11]. The administration of FLT3 or G-CSF increased the number of neutrophils. FLT3 and G-CSF are known to be important mediators of hematopoietic recovery and are important effectors in accelerating neutrophil recovery after radiation induced hematopoietic injury [10, 11]. In addition, it is known that FLT3 and G-CSF do play a role in protecting animals from the detrimental effects of radiation [14-16]. Previous studies have provided a rationale for using FLT3 concentration as a biological indicator of residual hematopoiesis after irradiation [16]. In this study, FLT3 increased in a dose dependent manner regardless of the dose rate. G-CSF also showed a dose dependent increase in mice irradiated with the low dose rate radiation; however, under the high dose rate radiation condition, no dose dependent increase was observed. In a previous study, low dose irradiation was shown to result in the production of the anti-inflammatory cytokine IL-10 and tissue damage in mouse skin [17]. The levels of proinflammatory cytokines, such as IL-2, IL-4, IL-5, IL-6, TNF-α and many growth factors varies with the dose-rate of irradiation [18,19].

However, no changes in the levels of other cytokines after irradiation were observed in this study. Only IL-6 level was elevated in high dose-rate 2 Gy radiation group. IL-6 is an important mediator that accelerates multilineage recovery after radiation induced hematopoietic aplasia mediated by G-CSF [20]. The change in IL-6 may be related to the prominent increase of G-CSF in the high dose rate group.

In this study, the precise molecular mechanism underlying the low dose rate of radiation remains unclear, but differential hematopoietic effects of radiation exposed at a high dose rate versus low dose rate were observed using the number of peripheral blood count and plasma cytokines. These data suggest that chronic low dose rate exposure caused a stimulation of hematopoietic system occurrence, unlike those observed after higher dose rate exposure. Our data suggest that the dose rate, rather than the total dose, may be more critical in causing damage to the cellular hematopoietic compartments of the body.

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