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^{28}Si total body irradiation injures bone marrow hematopoietic stem cells via induction of cellular apoptosis

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ABSTRACT

Long-term space mission exposes astronauts to a radiation environment with potential health hazards. High-energy charged particles (HZE), including ^{28}Si nuclei in space, have deleterious effects on cells due to their characteristics with high linear energy transfer and dense ionization. The influence of ^{28}Si ions contributes more than 10% to the radiation dose equivalent in the space environment. Understanding the biological effects of ^{28}Si irradiation is important to assess the potential health hazards of long-term space missions. The hematopoietic system is highly sensitive to radiation injury and bone marrow (BM) suppression is the primary life-threatening injuries after exposure to a moderate dose of radiation. Therefore, in the present study we investigated the acute effects of low doses of ^{28}Si irradiation on the hematopoietic system in a mouse model. Specifically, 6-month-old C57BL/6 J mice were exposed to 0.3, 0.6 and 0.9 Gy ^{28}Si (600 MeV) total body irradiation (TBI). The effects of ^{28}Si TBI on BM hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) were examined four weeks after the exposure. The results showed that exposure to ^{28}Si TBI dramatically reduced the frequencies and numbers of HSCs in irradiated mice, compared to non-irradiated controls, in a radiation dose-dependent manner. In contrast, no significant changes were observed in BM HPCs regardless of radiation doses. Furthermore, irradiated HSCs exhibited a significant impairment in clonogenic ability. These acute effects of ^{28}Si irradiation on HSCs may be attributable to radiation-induced apoptosis of HSCs, because HSCs, but not HPCs, from irradiated mice exhibited a significant increase in apoptosis in a radiation dose-dependent manner. However, exposure to low doses of ^{28}Si did not result in an increased production of reactive oxygen species and DNA damage in HSCs and HPCs. These findings indicate that exposure to ^{28}Si irradiation leads to acute HSC damage.

1. Introduction

Space irradiation is an unavoidable complication for astronauts during long-term space missions. Space irradiation is mainly composed of photons, protons, helium and high-energy charged particles (HZE). Eighty-eight percent of the space radiation dose is ascribed to HZE particles, such as ^{56}Fe, ^{28}Si, ^{16}O and ^{12}C (Cucinotta et al., 2003). Depending on dose, dose rate, track structure and fluency, different radiation sources have distinct biological effects on normal tissues. Among HZE particles, the influence of ^{28}Si ions contribute more than 10% to the radiation dose equivalent in space irradiation. Understanding the biological effects of ^{28}Si irradiation is needed and can benefit the development of new strategies to prevent and/or mitigate space radiation-induced injury.

The sensitivities of tissues to radiation progress from hematopoietic to gastrointestinal tissues and finally to neural and cardiovascular tissues as a function of increasing radiation doses. This indicates that the hematopoietic system is the most radiosensitive tissue of the body (Shao et al., 2014b). For example, low doses of 0.25–3 Gy proton irradiation significantly decreased the number of whole blood cells (WBCs) as early as 4 hours post exposure (Luo-Owen et al., 2012). Our recent data also showed that 1.0 Gy of proton total body irradiation (TBI) dramatically reduced the number of hematopoietic stem cells (HSCs) in bone marrow (BM) 22 weeks after irradiation (Chang et al., 2015). These results suggest that proton radiation induces both the acute and long-term

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damage to BM and BM HSCs. However, the effects of HZE particles (such as ^{28}Si) on the hematopoietic system, particularly BM HSCs, have not been reported.

A few studies reported the toxicities of ^{28}Si irradiation in cultured cells and animals. Daila et al. evaluated the effects of ^{28}Si irradiation on immune cells at 113 days after exposure, showing that 2.0 Gy of ^{28}Si irradiation decreased the numbers of nature killer cells and DNA synthesis (Gridley et al., 2002). Montree et al. recently reported that various doses of ^{28}Si irradiation triggered persistent cellular apoptosis in total bone marrow and heart tissues (Tungjai et al., 2013). Other *in vivo* studies have reported that low doses (0.25–1.0 Gy) of ^{28}Si irradiation adversely affected the central nervous system in mice, which led to the abnormalities in synaptic plasticity and cognitive function (Raber et al., 2014). In addition, using *in vitro* cell culture models, a few reports have shown that different doses and energies of ^{28}Si (0.07–6.0 Gy, 300–1000 Mev/nucleon) caused cellular chromosomal instability, cell transformation, decreased cell clonogenic survival and increased DNA damage in bronchial epithelial cells, esophageal epithelial cells, skin fibroblast and hamster embryo cells (Asaithamby et al., 2008; Ding et al., 2013; Tsuruoka et al., 2008, 2005). Both *in vitro* and *in vivo* data displayed the deleterious effects of ^{28}Si irradiation exposure on various types of cells and tissues. As the effects of ^{28}Si irradiation on HSCs and hematopoietic progenitor cells (HPCs) have not previously been reported, they were investigated in the present study.

2. Materials and methods

2.1. Animals and irradiation

Six-month-old male C57BL/6 J mice purchased from the Jackson Laboratory (Bar Harbor, ME) were shipped to Brookhaven National Laboratories (BNL) in Upton, NY. After a one-week acclimation period, the mice were either sham irradiated or received whole-body irradiation (600 MeV/n; 0.3, 0.6 0.9 Gy). One week after irradiation, the mice were shipped to Oregon Health and Science University (OHSU). At BNL and OHSU, the mice were housed under a constant 12 h light: dark cycle. Food (PicoLab Rodent Diet 20, no. 5053; PMI Nutrition International, St. Louis, MO) and water were provided *ad libitum*. Behaviorally naïve mice were used for experiments and analyzed at four weeks after irradiation. All procedures were approved by the Institutional Animal Care and Use Committee at OHSU and BNL.

2.2. Isolation of BM mononuclear cells (BM-MNCs), analysis of the frequencies and numbers of different hematopoietic cell populations by flow cytometry

The femora and tibiae were harvested from mice immediately after they were killed by cervical dislocation. BM cells were flushed from the bones into HBSS containing 2% FCS using a 21-gauge needle and syringe. Cells were then incubated with biotin-conjugated anti-CD3e, anti-CD45R/B220, anti-Gr-1, anti-CD11b, and anti-Ter-119 antibodies and with anti-CD16/32 (Fcγ II/III Receptor or FcγR) antibody to block the Fcγ receptors. They were labeled with streptavidin–FITC, anti-Sca-1–PE-Cy7, anti-c-Kit–APC–Cy7 for HPCs (Lin$^-$Sca1$^-$c-kit$^+$ cells), LSK cells (Lin$^-$Sca1$^+$c-kit$^+$ cells), and HSCs (Lin$^-$Sca1$^+$c-kit$^+$CD150$^+$CD48$^-$ cells). Bone marrow mononuclear cells (BM-MNCs) were isolated by Histopaque 1083 separation solution (Sigma, St. Louis, MO). For the isolation of lineage negative cells (Lin$^-$ cells), BM-MNCs were incubated with purified rat antibodies specific for murine CD3e, Mac-1, CD45R/B220, Ter-119, and Gr-1. The labeled mature lymphoid and myeloid cells were depleted by incubating with goat anti-rat IgG paramagnetic beads (Life Technologies, Grand Island, NY) at a bead: cell ratio of approximately 4:1. Cells binding the paramagnetic beads were removed with a magnetic field. Lin$^-$ cells were washed twice with 2% FBS/HBSS and re-suspended in complete medium (RPMI1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 10 μM HEPES buffer, and 100 U/mL penicillin and streptomycin) at 1×10^7 cells/mL. Subsequently, cells were blocked by Fcγ receptors anti-CD16/32 antibody then stained with anti-Sca1-PE-Cy7, c-Kit-APC–Cy7. All flow antibodies were purchased from eBioscience (San Jose, CA). The frequencies of HPCs and HSCs were analyzed with an Aria II cell sorter. Dead cells were excluded by gating out the cells stained positive with propidium iodide (PI). For each sample, approximately 8×10^5 to 1×10^6 BM cells were acquired and the data were analyzed using BD FACSDiva 6.0 (BD Biosciences) and FlowJo (FlowJo, Ashland, OR) software.

2.3. Colony-forming cell (CFC) assay and cobblestone area-forming cell (CAFC) assay

The CFC assay was performed by culturing BM-MNCs in MethoCult GFM3434 methylcellulose medium (Stem Cell Technologies, Vancouver, BC). Colonies of CFU–granulocyte macrophage (GM) were scored on day 7 and those of CFU-granulocyte, -erythrocyte, -monocyte, and -megakaryocyte (GEMM) on day 12 of the incubation according to the manufacturer's protocol. The CAFC assay was performed as described elsewhere (Li et al., 2011).

2.4. Analysis of the levels of intracellular reactive oxygen species (ROS)

Briefly, after staining with the appropriate cell surface marker antibodies, Lin$^-$ cells (1×10^7/mL) were suspended in PBS supplemented with 5 mM glucose, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$, and 5 mg/ml BSA and then incubated with 10 μM 2′,7′-dichlorofluorescin diacetate (DCFDA) (Life Technologies) for 30 minutes at 37 °C. The levels of ROS in HPCs and HSCs were analyzed by measuring the mean fluorescence intensity (MFI) of 2′,7′-dichlorofluorescein (DCF) with an Aria II cell sorter. For each sample, a minimum of 200,000 lineage negative cells was acquired and the data were analyzed as we previously described (Shao et al., 2014a).

2.5. DNA damage analysis

Lin$^-$ cells were first stained with antibodies against various cell-surface markers and fixed and permeabilized using the Fixation/Permeabilization Solution from BD Biosciences (San Diego, CA) followed by 0.2% Triton-X-100 incubation for 10 min. Cells were then stained with Alexa Fluor 488 conjugated anti-phospho-Histone 2AX (Ser139) antibody for 1.5 h at 4 °C and analyzed by flow cytometry. The levels of DNA damage were expressed by the mean fluorescence intensity of phospho-Histone 2AX or γ-H2AX with an Aria II cell sorter.

2.6. Cell cycle analysis

Lin$^-$ cells were first stained with antibodies against various cell-surface markers and fixed and permeabilized using the Fixation/Permeabilization Solution (BDBiosciences, San Diego, CA). Subsequently, they were stained with anti-Ki67-FITC antibody (BDBiosciences, San Diego, CA) and 7-AAD (Sigma, St. Louis, MO) and then analyzed by flow cytometer.

2.7. Statistical analysis

All data are presented as mean ± standard derivation of at least five independent biological samples per radiation dose. The differences between sham-irradiated and irradiated groups were examined by one way ANOVA, followed up by post-hoc test as indicated. Differences were considered significant at $p < 0.05$. Statistical analysis was performed using GraphPad Prism software (GraphPad Software Inc. LaJolla, CA).