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# Acute hematological effects in mice exposed to the expected doses, dose-rates, and energies of solar particle event-like proton radiation

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ABSTRACT

NASA has funded several projects that have provided evidence for the radiation risk in space. One radiation concern arises from solar particle event (SPE) radiation, which is composed of energetic electrons, protons, alpha particles and heavier particles. SPEs are unpredictable and the accompanying SPE radiation can place astronauts at risk of blood cell death, contributing to a weakened immune system and increased susceptibility to infection. The doses, dose rates, and energies of the proton radiation expected to occur during an SPE have been simulated at the NASA Space Radiation Laboratory, Brookhaven National Laboratory, delivering total body doses to mice. Hematological values were evaluated at acute time points, up to 24 hours post-radiation exposure.



## 1. Introduction

Cells with rapid turnover are most susceptible to the adverse effects of ionizing radiation, e.g., gastrointestinal cells, hematopoietic cells and reproductive cells. Hematopoietic cells are of interest because decreased blood cell counts leave irradiated individuals susceptible to infection and decreased immunity. Crew members during space flight are also at risk of developing problems from reduced numbers of peripheral blood cells caused by exposure to space radiation. Space radiation consists of particles trapped in the Earth's magnetic field, particles (primarily protons) originating from our Sun and galactic cosmic rays, which are high-energy protons and heavy ions from outside our solar system. The amount of space radiation an astronaut receives depends on several factors, including the location of the astronaut in the altitude above the Earth where shielding from the magnetic field is weaker. During a Solar Particle Event (SPE), significant spikes in the energy and fluence of solar particles increase the risk of astronaut exposure to higher doses of ionizing radiation.

SPEs are unpredictable, with more frequent events at the height of the 11 year solar cycle. SPEs, consisting of flares and coronal mass ejections, eject large amounts of high-energy protons at different dose rates. The dose-rates during an SPE are expected to vary from 10 to 50 cGy per hour (dependent on shielding). The August 1972 SPE is usually referred to as a worst-case scenario, with an omnidirectional proton fluence of $5.00 \times 10^9$ protons/cm$^2$ at energies above 30 MeV. If astronauts had been exposed to radiation from this SPE during extravehicular activity, the estimated total dose to the blood forming organs from this particular SPE would have been up to 1.38 Gy-Eq (Hu et al., 2009). It is important to note that SPE radiation is predicted to produce a highly inhomogeneous dose distribution in humans with external doses that are significantly higher than internal doses (Wilson et al., 1997). This raises several issues when attempting to model SPE-like radiation in mice in that the dose distribution (external > internal), energy/fluence and linear energy transfer (LET) spectrum cannot be simultaneously matched due to the relative size of humans and mice.

Previous reports on blood cell counts after proton radiation exposure include 1 GeV proton exposures, resulting in decreased white blood cell (WBC) and lymphocyte counts 24 hours after exposure (Wambi et al., 2009; Ware et al., 2010), as well as 24 hours after 70 MeV proton exposure (Maks et al., 2011) and 36 hours after 70 MeV proton exposure (Gridley et al., 2011; Luo-Owen et al., 2012). Blood cell counts in mice remained decreased 4 days and 21 days after exposure to 230 MeV protons (Gridley et al., 2008). In the present study we investigated the effect of simulated SPE proton radiation, producing an inhomogeneous dose distribution, in the mouse model. A homogeneous spread out Bragg peak proton beam was also utilized in this study to compare the effects of inhomogeneous simulated SPE proton radiation to homogeneous proton radiation on hematologic toxicity

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in the mouse model. The effects on hematopoietic cells at acute time points of 24 hours and as early as 4 hours after a single exposure to protons were evaluated, since the biological effects of exposure to significant doses of radiation are expected to manifest within hours of radiation exposure.

## 2. Materials/methods

### 2.1. Animals

Female ICR mice (5–7 weeks of age) were purchased from Taconic Farms, Inc. (Germantown, NY). Mice were housed 4 per cage under standard husbandry conditions with *ad lib* access to normal rodent chow and water. Upon arrival, the animals were acclimated for 7 days in the Brookhaven National Laboratory (BNL) Animal Facility. All protocols in the experiment were approved by the Institutional Animal Care and Use Committees (IACUCs) of the University of Pennsylvania and BNL.

### 2.2. Physics and dosimetry

Proton irradiations were performed at the NASA Space Radiation Laboratory (NSRL) at BNL. To deliver a dose distribution with consistent linear energy transfer, 8 different energies were chosen between 30.63 MeV and 74.62 MeV (referred to as 30–74 MeV throughout) to produce eight individual Bragg curves, which add up to an approximation of a flat dose distribution. The maximum proton energy of 74.62 MeV has a projected range in water of 4.57 cm; this energy was chosen to ensure that the dose to the mouse was homogeneous regardless of mouse orientation within the radiation chamber. To equalize the dose received by the animals during the irradiation and avoid any positional effect, the exposure was divided into 12.5 cGy segments in which the beam scrolled through the 8 different energies while delivering a total dose of 12.5 cGy. The mice were rotated 180 degrees and the process repeated until the required dose was delivered. Experiments and results from this proton radiation beam will be referred to as 30–74 MeV proton beam.

A separate experiment simulating an SPE was also performed at NSRL. The mouse SPE proton protocol required "scaling down" of the typical energies of an estimated SPE exposure. The energy distribution of the generic SPE is proportional to $e^{(-T/75)}$, where $T$ is the beam kinetic energy expressed as MeV. The energy distribution of the mouse SPE is proportional to $e^{(-(T-21.5)/19)}$. Energies ranging from 30 MeV to 150 MeV were delivered using 13 separate setup files, with each file programming the appropriate thickness of degrader filter to generate 13 different energies. The files were called up automatically, each with the calculated dose fraction to deliver a dose with an energy distribution approximating the scaled down mouse SPE. The energies, dose fraction, cutoff error and error fraction are listed in Table 1. Normalization values were calculated at the ionization chamber measurement point (depth = 1.27 mm) which was used at NSRL to calibrate each filtered beam component. Experiments and results relating to this proton radiation beam will be referred to as the scaled SPE proton beam.

### 2.3. Radiation exposure

Animals were placed in aerated radiation chambers (7.3 cm × 4.1 cm × 4.1 cm). Non-anesthetized mice were exposed to total body irradiation (TBI) in a single fraction at doses of 25, 50 cGy, 75 cGy, 100 cGy or 200 cGy at either a low dose rate (LDR) of 50 cGy/h or high dose rate (HDR) of 50 cGy/min. A separate experiment was performed using a very low dose rate of 0.28 cGy/min (17 cGy/h) at doses of 50, 75, or 100 cGy. For all proton radiation experiments, the mouse cages were rotated 180 degrees every 1/4 of the total dose at the low dose rate and 1/2 of the total dose at the high dose rate.

**Table 1**
Energy distribution and energy degrader filter settings of the simulated SPE exposure with an overall error from cutoff less than 1%.

| MeV | Filter setting (cm) | Dose fraction (%) | Cutoff error (%) | Error fraction |
|---|---|---|---|---|
| 30 | 14.325 | 92.3480 | 1 | 0.0092 |
| 40 | 13.75 | 3.6400 | 1 | 0.000036 |
| 50 | 13.05 | 1.8131 | 2 | 0.000036 |
| 60 | 12.225 | 0.9882 | 5 | 0.00049 |
| 70 | 11.275 | 0.5203 | 10 | 0.0005 |
| 80 | 10.20 | 0.2997 | 10 | 0.0003 |
| 90 | 9.050 | 0.1646 | 10 | 0.0002 |
| 100 | 7.775 | 0.0997 | 10 | 0.0001 |
| 110 | 6.400 | 0.0536 | 10 | 0.00005 |
| 120 | 4.925 | 0.0295 | 10 | 0.00003 |
| 130 | 3.375 | 0.0181 | 10 | 0.00002 |
| 140 | 1.725 | 0.0103 | 10 | 0.00001 |
| 150 | 0.000 | 0.0061 | 10 | 0.000006 |

To adequately compare the LDR data to the HDR data or the extremely low dose rate data to the HDR data, all animals were confined in the radiation chamber for the complete LDR exposure time. For example, the group of animals exposed to the 25 cGy dose at the HDR were only exposed for 0.5 min, but remained in the radiation chamber for a complete confinement time of 30.0 min, which mimics the 25 cGy LDR exposure time. Additionally, the non-irradiated control groups were also confined in the irradiation chambers for the longest duration of the radiation exposure times (4 hours total). Separate sham-irradiated control groups remained in the irradiation chamber for approximately 3, 4.5, or 6 hours to mimic the radiation exposure time of the mice exposed to the very low dose rate (0.28 cGy/min) at doses of 50, 75, or 100 cGy. To eliminate diurnal variation in the hematology analyses, the exposure start times were staggered so that all dose groups were removed from the beam at the same time and post-radiation procedures were performed all together at the appropriate time point(s).

### 2.4. Hematopoietic cell count analyses

At 4 or 24 hours after completion of the exposure, 8–12 mice irradiated at each radiation dose and dose rate were euthanized by $CO_2$ asphyxiation followed by cardiac puncture to collect blood. For the exposures at the very low dose rate of 0.28 cGy/min, blood was collected only at the 24 hour time point. The blood from each animal was collected and placed into a lavender top blood collection tube containing EDTA and stored at ambient temperature. The blood samples were analyzed by a Bayer Advia 120 Hematology Analyzer (Antech Diagnostics, Lake Success, NY) and a complete blood cell count with differential was performed within 24 hours of blood collection.

### 2.5. Statistical analyses

The average counts of white blood cells (WBCs), neutrophils, and lymphocytes were determined in the sham-irradiated controls as baseline values. The blood cell counts obtained in animals at different time points were divided by the respective baseline values and expressed as fractions of control for statistical analyses. Histograms were generated using GraphPad Prism software (Version 5) and data analyzed by the Student's T test, comparing each dose point to the 0 cGy controls or comparing the results obtained from each dose-rate experiment at the same dose point.

The dose effect on the blood cell counts was determined by variance analyses using a general linear model, which was performed using a Minitab statistical software, release 15 (Minitab

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