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Comparison of changes over time in leukocyte counts in Yucatan minipigs irradiated with simulated solar particle event-like radiation

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ABSTRACT

During a major solar particle event (SPE), astronauts in space are at risk of exposure to an increased dose of proton radiation. The whole body distribution of the absorbed SPE proton dose is inhomogeneous, and such an inhomogeneous SPE proton dose can be simulated by electron radiation. Using Yucatan minipigs as an animal model, we compared the time courses of leukocyte count changes after exposure to proton simulated SPE (pSPE) radiation or electron simulated SPE (eSPE) radiation. The results demonstrated that the time required after irradiation to reach the lowest leukocyte counts was generally comparable between the pSPE and eSPE radiation exposures. However, the leukocyte count often recovered faster after electron irradiation compared to proton irradiation at the corresponding doses. In addition, the radiation dose required to achieve comparable magnitudes of leukocyte count decrease was higher in the eSPE animals than for the pSPE animals. In conclusion, based on the magnitude of the decrease and the time required to reach the lowest leukocyte counts after irradiation, the pSPE radiation was more effective than the eSPE radiation in reducing the peripheral leukocyte counts. Lymphocytes appeared to be the most sensitive type of leukocytes in response to either type of SPE radiation. It is particularly noteworthy that following exposure to pSPE radiation at the skin doses >5 Gy, the neutrophils do not recover from the radiation damage at times up to 30 days, and the neutrophils have not recovered to their baseline levels even at 90 days post-irradiation. These results suggest a marked difference in the ability of the neutrophils to recover from pSPE radiation compared with the results observed for eSPE radiation.

1. Introduction

During a major solar particle event (SPE), astronauts in space are potentially at significant risk of increased radiation exposure, which could result in acute radiation sickness, skin injury, and/or a compromised immune defense. SPEs originate from magnetically disturbed regions of the Sun, which sporadically emit bursts of energetic charged particles (Wilson et al., 1999; Smart and Shea, 2003). Large SPEs are expected to occur only once or twice in a solar cycle; however, during the 22nd solar cycle, four extremely large SPEs of comparable magnitude occurred within a 4-month period. The types of radiation emitted during SPEs are predominately protons (Hellweg and Baumstark-Khan, 2007). The majority of protons in an SPE are at or below 50 MeV, but each event varies in energy spectra and proton fluence. At these lower energies, the distribution of the absorbed proton dose received during an SPE is expected to be inhomogeneous, with a significantly higher absorbed dose delivered to the skin and subcutaneous tissues than to the internal organs (Coutrakon et al., 2007; Hu et al., 2009). The inhomogeneous dose distribution makes it difficult to determine the effectiveness of the SPE radiation relative to the effect of conventional radiation commonly available on Earth for radiation biology research. To overcome this problem, a novel concept of using megavoltage electron beam radiation to simulate the total dose and dose distribution of SPE protons has been explored in animal models (Cengel et al., 2010). Electron radiation with mixed energies of 6 MeV + 12 MeV (6 + 12 MeV), which simulate the whole body dose distribution of SPE radiation (Cengel et al., 2010), can be used as a reference radiation to determine the relative biological effectiveness (RBE) of proton simulated SPE radiation.

The doses used in our studies to mimic SPE radiation exposure in animal studies have utilized relatively high doses of radiation that astronauts could have received from exposure to SPE radiation during historical SPEs. The total skin doses estimated for possible astronaut exposure to SPE radiation during extravehicular activity,

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as predicted from modeling 3 different historical SPEs, would have been >5 Gy (specific estimations for total skin doses were as follows for these 3 SPEs: 7.68, 25.99 and 32.15 Gy) (Hu et al., 2009). It is recognized that these levels of exposure to SPE radiation would represent “worst-case” scenarios, and that the probability of astronaut exposure to skin doses of this magnitude is very low (Hu et al., 2009). The SPE-like radiation skin doses used in the studies reported here are within these worst-case scenario skin doses estimated for potential astronaut exposure to SPE radiation.

We have previously evaluated the dose responses of SPE-like proton radiation in Yucatan minipigs (Sanzari et al., 2013a). Using 6 + 12 MeV electrons as a reference radiation, we have also determined the RBE for the SPE-like proton radiation with respect to the hematological effects in Yucatan minipigs (Sanzari et al., 2014). In the present study, the minipig peripheral leukocyte count data obtained from the proton radiation study (Sanzari et al., 2013a) and the 6 + 12 MeV electron radiation study (Sanzari et al., 2014) were analyzed to compare and contrast the time course differences between the hematological effects of the SPE-like proton radiation and 6 + 12 MeV electron radiation.

2. Materials and methods

2.1. Animals

As previously described (Sanzari et al., 2013a, 2014), male Yucatan minipigs aged 8–12 weeks were purchased from Sinclair Bio Resources, LLC (Auxvasse, MO). The animals were housed individually with ad lib access to water and fed standard chow twice daily. After acclimation for 7 days, the animals were randomized into different treatment groups with 3 animals per group and exposed to proton simulated SPE radiation at skin doses of 5, 7.7 and 10 Gy or electron simulated SPE radiation at skin doses of 5, 7.5, 10 and 20 Gy.

The animal care and treatment procedures were approved by the Institutional Animal Care and Use Committee of Loma Linda University Medical Center (LLUMC) and the University of Pennsylvania (Penn).

2.2. Irradiation

The radiation experiments with SPE-like protons and 6 + 12 MeV electrons were described in detail previously (Sanzari et al., 2013a, 2014). For the proton radiation experiment, the animals were exposed to beams comprised of protons with energy distribution up to 155 MeV and a custom depth dose profile similar to what is expected for SPE radiation; specifically, the August 1989 SPE. The source, modulation and characterization of the proton beams, as well as the proton dose calibration, delivery and monitoring were described in detail previously (Sanzari et al., 2013c). For the electron radiation experiment, the animals were irradiated with 6 + 12 MeV electron beams composed of a mixture of 80% 6 MeV electrons and 20% 12 MeV electrons, which closely mimics the whole body dose distribution of the August 1989 SPE (Diffenderfer et al., 2014). Throughout the remainder of manuscript, proton simulated SPE radiation and electron simulated SPE radiation will be referred to as pSPE and eSPE, respectively.

2.3. Blood cell count analyses

Prior to irradiation and at 4 hours and 1, 4, 14, 30 and 90 days after the proton irradiation or 4 hours and 1, 7, 14, 30 and 85 (for all but the 5 Gy electron dose group, in which the time point at 85 days was not collected) days after electron irradiation, a whole blood sample was collected from each animal (cranial vena cava). The blood samples (maintained at 4 °C) were sent to Antech Diagnostics (Irvine, CA) and analyzed using a Bayer Advia 120 Hematology Analyzer within 24 hours of the blood sample collection, as previously described (Sanzari et al., 2013a, 2014). It has been previously determined that the blood cell counts are stable and reliable when comparing blood cell counts analyzed immediately after collection or within/up to 24 hours after collection utilizing the automated analyzer used in this study (Romero-Weaver and Kennedy, 2012).

2.4. Data and statistical analyses

The minipig peripheral leukocyte count data obtained from the proton radiation study (Sanzari et al., 2013a) and the 6 + 12 MeV electron radiation study were analyzed to compare and contrast the time course differences between the hematological effects of the SPE-like proton radiation and 6 + 12 MeV electron radiation. The mean counts of WBCs, lymphocytes, neutrophils, monocytes and eosinophils of all animals prior to the radiation exposure were calculated and used as the baseline values for the respective blood cell types in the same experiment. For each animal at each time point after irradiation, the count of each blood cell type was divided by the respective baseline value, and the result was expressed as fraction of control for further analyses.

The mean results and standard errors for WBCs, lymphocytes, neutrophils, monocytes and eosinophils were calculated for each treatment group at each time point. The mean results for each type of leukocytes were plotted against the time (days) elapsed after radiation exposure to illustrate the time course of leukocyte count changes after irradiation. The results for the different time points after irradiation were analyzed by one-way ANOVA followed by the Holm–Sidak test for a comparison with the respective baseline values.

3. Results

The time courses for the change in the leukocyte counts were compared between Yucatan minipigs exposed to pSPE and eSPE radiation; eSPE radiation was used as the reference radiation. In the minipigs exposed to the pSPE radiation, the WBC count decreased significantly by 54.2% ($p < 0.01$) on day-4 after irradiation at a single dose of 5 Gy and by 57.4% ($p < 0.01$) and 59.3% ($p < 0.01$) on day-1 after irradiation at a single dose of 7.7 and 10 Gy, respectively (Fig. 1A).

The WBC count recovered slowly thereafter. By day-30 after irradiation, the WBC count for the pSPE irradiated groups remained 17.5% ($p = 0.57$) to 38.3% ($p < 0.05$) below the baseline level, although the decrease was only significant for the 7.7 Gy pSPE dose group. By day-90 after the pSPE irradiation, the WBC count recovered fully in the 5 Gy pSPE dose group, but was still 18.7% ($p = 0.20$) and 33.5% ($p = 0.11$) below, although no longer significantly different from, the baseline value for the 7.7 and 10 Gy pSPE dose groups, respectively. Despite the observed changes in the differential blood cell counts, all animals were reported stable upon a daily health check post-irradiation and throughout the experimental period. The daily health check included inspection of eyes, skin, overall body wellness and breathing, responsiveness to socialization, food check, and water intake.

In the animals exposed to eSPE radiation, the WBC count for the 5 Gy electron dose group did not decrease significantly after irradiation, except for day-30 after irradiation when the WBC count decreased by 44.1% ($p < 0.05$) as compared with the baseline level (Fig. 1B). In the 7.5 Gy eSPE dose group, the WBC count decreased significantly by 54.9% ($p < 0.05$) within a day after irradiation, then recovered quickly within 7 days after irradiation to a level that was no more than 24.0% below the baseline value ($p \geq 0.53$).