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Analysis of White Blood Cell Counts in Mice after Gamma- or Proton-Radiation Exposure

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Abstract

In the coming decades human space exploration is expected to move beyond low-Earth orbit. This transition involves increasing mission time and therefore an increased risk of radiation exposure from solar particle event (SPE) radiation. Acute radiation effects after exposure to SPE radiation are of prime importance due to potential mission-threatening consequences. The major objective of this study was to characterize the dose–response relationship for proton and γ radiation delivered at doses up to 2 Gy at high (0.5 Gy/min) and low (0.5 Gy/h) dose rates using white blood cell (WBC) counts as a biological end point. The results demonstrate a dose-dependent decrease in WBC counts in mice exposed to high- and low-dose-rate proton and γ radiation, suggesting that astronauts exposed to SPE-like radiation may experience a significant decrease in circulating leukocytes.

INTRODUCTION

In the coming decades human space exploration is expected to move beyond low-Earth orbit. This transition involves increasing mission time and therefore an increased risk of radiation exposure from both galactic cosmic rays (GCR) and solar cosmic radiation (SCR). An important component of SCR is the solar particle event (SPE) that occurs when highly charged particles are released from magnetically disturbed regions of the Sun (1). SPE radiation consists mostly of protons, with a small component of heavy ions (2). SPEs are unpredictable, develop rapidly and can last for several hours to several days. Not only is the timing of an event unpredictable, but the spectrum of proton energies differs from one event to the next, making the dose distribution delivered to the tissue very difficult to determine (2).

In a previous study, three historically large SPEs (August 1972, October 1989 and September 1989) were modeled to determine the theoretical doses and dose rates for an astronaut on extravehicular activity (EVA) during these SPEs (2). Dose estimates to blood-forming organs during these three historical SPEs ranged from 37 cGy to 1.38 Gy for

astronauts during EVAs, while doses to the skin were estimated to be as high as 32 Gy during EVA (2). In this range, symptoms of early morbidity similar to a mild acute radiation syndrome could be possible and would have the potential to degrade crew performance and interfere with mission success.

In addition to the late effects that could be caused by space radiation exposure, including cancer, cataracts and neuronal disease, acute effects are of prime importance due to potential mission-threatening consequences. The major objective of this study was to characterize the dose–response relationship of 50 and 70 MeV/n proton and γ radiation delivered at doses up to 2 Gy at high (0.5 Gy/min) and low (0.5 Gy/h) dose rates using white blood cell (WBC) counts as a biological end point. The variables in this study were radiation type, proton energy, dose and dose rate. In addition to determining the dose–response relationships, relative biological effectiveness (RBE) values were determined with γ radiation as the reference radiation.

MATERIALS AND METHODS

Animals

Female ICR outbred mice aged 5–6 weeks were obtained from Harlan Laboratories (Livermore, CA) and given an acclimation period of 7 days at the Loma Linda University Medical Center (LLUMC). Five to six animals were housed per cage with *ad libitum* access to food and water with a 12-h light-dark cycle. The animals were maintained on a normal rodent chow diet under standard husbandry conditions. All procedures for animal care and treatment were approved by the Institutional Animal Care and Use Committees (IACUCs) of the University of Pennsylvania and the LLUMC.

Physics and Dosimetry

Gamma radiation was chosen as the reference radiation for the determination of RBE values and was delivered using a ^{60}Co source (Eldorado Model ‘G’ machine, Atomic Energy of Canada Ltd., Commercial Products Division, Ottawa, Canada) at the LLUMC. For radiation exposures at the high dose rate, the source-to-target distance was 150 cm, with a usable radiation field of $40 \times 40 \text{ cm}^2$ and a field flatness of 5.4% in the horizontal direction and 3.6% in the vertical direction. No additional material was placed between the source and target to modify the dose rate. For low-dose-rate irradiations, the source-to-target distance was 195 cm with a usable field size of $40 \times 40 \text{ cm}^2$ and a field flatness of 4.0% in the horizontal direction and 2.3% in the vertical direction. To achieve the low dose rate, an additional 12.7 cm of steel plating was placed between the source and the target to attenuate the flux of the beam. Depth dose measurements were made using a calibrated Markus ionization chamber for comparison with proton irradiations (Fig. 1).

A whole-body homogeneous dose distribution was essential for both γ and proton radiation for accurate RBE calculations. ^{60}Co provides a fixed depth dose relationship that is largely homogeneous across the sample volume, while the proton depth dose curve can be modified according to the proton energy, modulator wheel and degrader selected. For 70 MeV/n exposures, a fully modulated proton beam was chosen to deliver a homogeneous dose distribution and allow for comparison to γ -ray exposures (Fig. 1) and RBE calculations. A fully modulated 70 MeV/n proton beam ensured delivery of a homogeneous dose, regardless of the mouse’s orientation within the cubicle, since the water equivalent range of a 70 MeV/n proton is approximately 4.1 cm. For both dose rates, a 70 MeV/n fully modulated proton beam was delivered at the inside of the mouse cage. This setup delivered a maximum proton energy of 70 MeV/n and was also comprised of a distribution of proton energies below 70 MeV/n [spread-out Bragg peak (SOBP)], which is reflective of the space environment where

the maximum proton energy is accompanied by a significant number of lower-energy protons.

The mouse chambers used during the radiation exposure were 4.13 cm deep, which allowed the animals to easily turn around in the chamber. Since a 50 MeV/n proton beam will deposit its Bragg peak at approximately 2.5 cm, a 50 MeV/n SOBP proton beam is not expected to penetrate the entire depth of the chamber; therefore, the dose delivered to various tissues in the mouse will depend on the position of the animal during the exposure and thus could potentially result in deposition of an inhomogeneous dose.

Both the 50 and 70 MeV/n proton exposures were performed in the horizontal clinical beam line at the LLUMC using an incident beam of 155 MeV/n. The incident protons were scattered into a uniform field using the clinical two-stage scattering system and modulated in depth using a 3- or 5-cm clinical modulator wheel for the 50 or 70 MeV/n exposure, respectively. Prior to entering the experimental setup, the beam was degraded to the required energy using a predetermined thickness of polystyrene. The high- and low-dose-rate experiments used two different source-to-target distances to achieve the desired dose rates and beam sizes, while the energy loss in air between the two setups was compensated using two different polystyrene degrader thicknesses. Depth dose profiles for the 70 MeV/n protons were measured for the optimized polystyrene degrader thickness using Gafchromic film, type MD-55, and verified using ion chamber measurements. The usable field size for the high-dose-rate setup was $14 \times 14 \text{ cm}^2$ for both proton energies with a field flatness in the horizontal and vertical directions of 7.0% or 3.0% for 50 and 70 MeV/n protons, respectively. In the case of the low-dose-rate setup, the usable field size was approximately $16 \times 16 \text{ cm}^2$ or $20 \times 20 \text{ cm}^2$ with a field flatness of approximately 7% or 8% for 50 or 70 MeV/n protons, respectively.

Proton and γ -Ray Exposure

After acclimation, the mice were placed in plastic chambers (AMAC no. 530C) with dimensions of $7.30 \text{ cm} \times 4.13 \text{ cm} \times 4.13 \text{ cm}$ (approved by the LLUMC IACUC). The chambers allowed the mice to easily turn around (reverse nose to tail direction). There were ample holes at the top of the chamber to allow for air flow and at the bottom to allow for drainage of feces and urine. The mice were exposed to total-body radiation with ^{60}Co γ rays at doses of 0.13, 0.25, 0.5, 1 and 2 Gy or SOBP protons (50 or 70 MeV/n) at doses of 0.25, 0.5, 1 and 2 Gy. All exposures were delivered in a single dose at the low dose rate (0.5 Gy/h) or high dose rate (0.5 Gy/min). The mouse cages were rotated 180° for every one-fourth of the total delivered dose for the low dose rate and half of the total delivered dose for the high dose rate.

Blood Cell Count Analyses

At 24 h after the completion of the radiation exposure, six mice irradiated at each radiation dose and dose rate were euthanized by CO_2 asphyxiation followed by cardiac puncture to collect blood. The blood from each animal was collected and placed into a lavender top collection tube containing EDTA and kept at ambient temperature. The blood samples were sent to Antech Diagnostics (Irvine, CA) and analyzed using a Bayer Advia 120 Hematology Analyzer at times up to 24 h after blood collection. A separate study aimed at comparing manual counts to automated counts at Antech Diagnostics demonstrated no statistically significant differences between the two methods of data collection or times at which the samples were processed from immediately postirradiation to 24 h postirradiation (unpublished data from A. Romero-Weaver, J. H. Ware, J. S. Sanzari and A. R. Kennedy).

Effect of Confinement

Since the 2-Gy dose at the low dose rate required 4 h of exposure time, the effect of confinement was evaluated in four independent experiments as a potential modifier of experimental results. Each experiment consisted of 5 or 6 mice placed in irradiation chambers for 2–4 h and 6 to 12 control mice kept in conventional cages with free access to food and water. Twenty-four hours after the mice were removed from the chambers and placed back in their home cages, blood samples were taken for analysis of WBC counts according to the methods described above.

Data and Statistical Analyses

WBC counts were divided by those for the appropriate control group from the same experiment to obtain the percentage of control for all cohorts. The WBC count data for different treatment groups were compared by one-way ANOVA followed by Tukey's test using GraphPad Prism statistical software (Version 5). The design of experiment (DOE) analysis, used widely in research and development for process improvement (3–9), was completed to allow for all experimental factors to be evaluated simultaneously (Minitab statistical software, Release 15).

The DOE analysis was performed on the WBC counts and the natural logarithmically transformed counts using a general linear model to simultaneously evaluate the effect of the variables used in this study on the biological end point in question. The plots of the DOE analysis showed that the residual distribution was random for the natural logarithmically transformed data but was skewed for the untransformed data (data not shown). To avoid a violation of the normality assumption, the DOE analysis was performed on the natural logarithmically transformed data.

The relationship between the dose and WBC count was determined by fitting the data to an exponential decay model ($y = ae^{-kx}$) using SigmaPlot graphics software (SPSS Inc., Chicago, IL), with x equal to dose and y equal to the WBC count. The radiation sensitivity constants (k) for WBC counts were solved for according to the above equation for each type of radiation, energy and dose rate. The dose of each radiation type required to yield 37% survival (known as the D_{37} , which equals $1/-k$) was determined and used to calculate the RBE value for 50 and 70 MeV/n protons relative to γ radiation ($D_{37(\gamma)}/D_{37(\text{protons})}$ or $-k_{(\text{protons})}/-k_{(\gamma)}$).

RESULTS

The comparison of WBC counts from cage-confined mice and untreated mice demonstrated no statistically significant differences (data not shown). These results indicate that confinement in cages used for radiation exposures for up to 4 h does not significantly affect the WBC counts when the blood samples were taken at 24 h after the mice were placed in the chamber. Thus separate control groups adjusting for confinement stress were not included in this study.

RBE Calculation for WBC Counts

Data collected from two 50 MeV/n proton experiments, three 70 MeV/n proton experiments, and two different sets of two γ -ray experiments were used for determination of the dose–response relationship and RBE values. The combined experiments indicate that exposure to high-dose-rate ^{60}Co γ radiation and high-dose-rate 70 MeV/n protons results in a dose-dependent decrease in WBC counts when assayed 24 h after irradiation (Fig. 2A). The threshold dose was defined as the lowest dose that exhibited a statistically significant reduction in cell counts compared to those observed for control mice. The threshold doses

for WBC loss in mice exposed to high-dose-rate γ radiation and high-dose-rate 70 MeV/n proton radiation were 0.5 Gy and 0.25 Gy, respectively. Low-dose-rate exposure to γ radiation and 70 MeV/n proton radiation similarly resulted in a dose-dependent decrease in WBC counts (Fig. 2B). Threshold doses for WBC loss in mice exposed to low-dose-rate γ radiation and low-dose-rate 70 MeV/n proton radiation were 0.25 Gy and 0.5 Gy, respectively.

The radiation sensitivity constants for WBCs in mice exposed to high-dose-rate ^{60}Co γ rays and high-dose-rate 70 MeV/n protons were 1.047 ($R^2 = 0.954$) and 1.1026 ($R^2 = 0.965$), respectively (Fig. 3A). These results indicate an RBE of 1.05 (1.103/1.047) ($P = 0.32$) for WBC loss in mice exposed to high-dose-rate 70 MeV/n protons (with the reference ^{60}Co γ rays administered at the high dose rate). The radiation sensitivity constants for WBCs in mice exposed to low-dose-rate ^{60}Co γ rays and low-dose-rate 70 MeV/n protons were 0.561 ($R^2 = 0.928$) and 0.617 ($R^2 = 0.9560$), respectively (Fig. 3B). These results indicate an RBE of 1.10 (0.617/0.561) ($P = 0.35$) for WBC loss in mice exposed to low-dose-rate 70 MeV/n protons (with the reference ^{60}Co γ rays administered at the low dose rate).

The radiation sensitivity constants for WBCs in mice exposed to high-dose-rate ^{60}Co γ rays and high-dose-rate 50 MeV/n protons were 0.632 and 0.339, respectively (Fig. 4A). These results indicate an RBE of 0.54 [0.339/0.632 ($P = 0.07$)] for WBC loss in mice exposed to high-dose-rate 50 MeV/n protons with reference radiation of ^{60}Co γ rays administered at the high dose rate]. The difference in radiation sensitivity constants was marginally significant ($P = 0.07$) between γ rays and 50 MeV/n protons at the high dose rate. The radiation sensitivity constants for WBCs in mice exposed to low-dose-rate ^{60}Co γ rays and low-dose-rate 50 MeV/n protons were 0.617 and 0.449, respectively (Fig. 4B). These results indicate an RBE of 0.73 (0.449/0.617) ($P = 0.36$) for WBC loss in mice exposed to low-dose-rate 50 MeV/n protons (with reference radiation of ^{60}Co γ rays administered at the low dose rate). There were no statistically significant differences between the radiation sensitivity constants of γ radiation compared to the 50 MeV/n protons ($P = 0.36$).

The statistical significance and relative importance of different experimental factors and their interactions were determined by a four-factorial DOE analysis of the data derived from two separate 70 MeV/n proton experiments and two separate γ -ray experiments performed at the same time as the proton experiments. The results of this DOE analysis and results are as follows:

1. 70 MeV/n protons compared to γ rays—Based on the DOE analysis of the naturally logarithmically transformed data, radiation type (70 MeV/n protons or γ rays) was not a significant influencing factor on WBC counts since the sum of squares (SS) attributable to radiation type accounted for less than 0.01% of the total SS for WBCs (Fig. 5B, $P = 0.11$). The SS represents the proportion of total SS observed in the experiment that can be explained by the experimental variable in question. For example, an SS of less than 0.01% indicates that the variable in question had virtually no influence on the biological end point measured. The statistical significance of the interaction between the radiation type and dose in the two-factorial DOE analyses described above indicates whether the radiation dose response is significantly different between the 70 MeV/n proton and γ -radiation exposures. Therefore, the P values for the interaction are also used to determine whether there were statistically significant differences between the results observed experimentally and those expected from a null hypothesis value of 1.

The effects of 70 MeV/n proton and γ radiation were not significantly different from each other in their ability to kill WBCs. Similarly, the RBE values for 70 MeV/n protons at both

dose rates for WBC counts were not significantly different from the null hypothesis value of 1.

2. Dose-rate effects—The WBC counts were slightly lower for the high-dose-rate exposures compared to the low-dose-rate exposures, and the difference was statistically significant (Fig. 5C, $P < 0.001$). However, the dose rate had only a minor influence on WBC counts in the experiment since the SS was only 11.19% of the total SS.

3. Radiation dose—The radiation dose (0.25, 0.5, 1 and 2 Gy) had by far the most influence on WBC counts, as expected, since the SS attributable to radiation dose accounted for 59.63% of the total SS (Fig. 5D, $P < 0.001$).

4. Experimental variation—Two complete and separate experiments were performed and the WBC counts for each paired experiment were analyzed by the DOE analysis. The results demonstrated that the mean WBC counts were lower in Experiment I than in Experiment II. The difference reached statistical significance, although the variation between the two experiments was only a minor influencing factor since the SS was negligible and accounted for only 0.20% of the total SS (Fig. 5A, $P = 0.01$).

DISCUSSION

Over the dose range investigated (0–2 Gy), γ rays and 70 MeV/n protons had similar effects on WBC counts, and the dose-rate effect and dose response obtained with γ rays were predictive of the dose-rate effect and dose response of 70 MeV/n proton radiation and vice versa. The DOE analysis did reveal a small but statistically significant dose rate effect for both 70 MeV/n protons and γ rays, with the high dose rate having a stronger effect on the WBC counts than that observed for the 70 MeV/n protons and γ radiation administered at the low dose rate. These results differ from some of the previously published data. For example, in two previous studies, the dose-rate effect on WBCs was evaluated by irradiating mice in the dose range of 1–3 Gy with 250 MeV/n protons at dose rates of 0.01 Gy/min and 0.8 Gy/min. The investigators did not observe a significant dose-rate effect on WBCs four days after the radiation exposure (10, 11). Ware *et al.* (12) investigated the dose-rate effect by irradiating mice with up to 2 Gy of 1 GeV/n protons at dose rates of 0.5 Gy/min and 0.05 Gy/min and also found no significant dose-rate effect on WBC counts 24 h after irradiation at doses of 0.5, 1, 1.5 and 2 Gy. The small but significant dose-rate effect on WBC counts observed in this study illustrates the statistical power of the DOE analysis and its ability to detect differences in complicated biological experiments not otherwise revealed with typical statistical methods. It is important to note that while the DOE analysis detected small differences in dose rate, there were similar dose-rate effects in both the γ -ray and proton groups of mice, demonstrating that the dose-rate effect is present for both radiation types.

The experimental results after irradiation with 50 MeV/n protons illustrate the importance of considering the animal model, dosimetry and proton energy to ensure delivery of a homogeneous dose, especially when calculating RBE values. No significant differences were observed in the radiation sensitivity constants for the low dose rate between γ rays and 50 MeV/n protons for WBC counts 24 h after irradiation. These results, along with the findings from the 70 MeV/n experiments, indicate that exposure of mice at proton energies above 50 MeV/n at the low dose rate result in similar biological effects. This is likely due to the mouse changing positions many times during the lengthy exposure period, resulting in randomization and a nearly homogeneous dose distribution.

The differences between the results observed for 50 MeV/n protons and γ radiation on WBC counts at the high dose rate were of borderline statistical significance ($P = 0.07$). For the

high-dose-rate exposures, the mouse likely changed positions only a few times during the relatively short exposure, resulting in an inhomogeneous dose distribution. This likely reduced the dose delivered to portions of the blood and blood-forming organs and resulted in the sparing of circulating leukocytes and blood-forming organs. The results presented here suggest that for high-dose-rate exposures, a minimum energy of 70 MeV/n is required to deliver a homogeneous dose. The dose-rate effect observed for 50 MeV/n proton radiation in these experiments results in the opposite effect of that observed for the 70 MeV/n experiments, since the high-dose-rate 50 MeV/n proton exposures resulted in a decreased RBE value and therefore a decreased effectiveness in reducing WBC counts compared to low-dose-rate 50 MeV/n proton exposures. Thus the dose-rate effect observed for 50 MeV/n proton in this study is most likely due to the inhomogeneous dose delivered during the 50 MeV/n proton exposures rather than being a result of radiation damage or radiation-induced adaptive responses, which are the current hypotheses for the dose-rate effect leading to sparing effects, which has been observed by other investigators (13–16).

While it is highly likely that dosimetry and not radiobiology is the root cause for the dose-rate effect observed in the 50 MeV/n proton experiments, this cannot be confirmed without additional studies. An exposure designed to deliver a homogeneous dose of high- and low-dose-rate 50 MeV/n protons would be necessary. To do so, however, the depth of the mouse cage would have to be reduced from 4.13 cm to 2.5 cm, making the study unethical and potentially harmful to the animals.

The purpose of this study was to characterize the dose–response relationship and dose-rate effect of relatively low-dose and low-energy proton radiation, which is the major component of SPE radiation. For the 70 MeV/n proton exposures, the data demonstrate a clear dose-dependent decrease in WBC counts as well as a small yet statistically significant dose-rate effect on WBC counts. Dose-rate effects of ionizing radiation have been a source of continued debate. Decreased cytotoxic effects have been described for low-LET radiation at reduced dose rates in both *in vivo* and *in vitro* systems; however, any sparing effect for high-LET radiation delivered at low dose rates remains unclear (17). This work demonstrates that dose rate is an influencing factor for WBC counts; however, the difference is small and is present for both γ -ray and proton exposures.

As the DOE analysis demonstrates, dose was the most significant factor for determining levels of WBC counts, as expected. Threshold doses for γ radiation and 70 MeV/n protons at both dose rates range from 0.25 to 0.5 Gy, with no apparent correlation with dose rate or radiation type. The RBE values for WBC counts after γ or 70 MeV/n proton irradiation at both dose rates, with γ radiation as the reference radiation, are not significantly different from the null hypothesis value of 1.

Conclusion

These results demonstrate a dose-dependent decrease in WBC counts in mice exposed to high- (0.5 Gy/min) and low- (0.5 Gy/h) dose-rate 70 MeV/n proton and γ radiation, suggesting that astronauts exposed to SPE-like radiation may experience a significant decrease in circulating leukocytes. Threshold doses range from 0.25 to 0.5 Gy for protons and γ radiation. Statistical analysis demonstrates a small (11.2%) dose-rate effect, with the low-dose-rate radiation being less effective for both γ rays and 70 MeV/n protons. Furthermore, a comparison of results for exposure to 70 MeV/n protons with γ radiation as the reference radiation results in RBE values that are not significantly different from 1 for WBC counts evaluated at 24 h after the radiation exposure.

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FIG. 1. Water equivalent depth dose distribution for 70 MeV/n protons and ^{60}Co γ rays at both the high dose rate and low dose rate.

FIG. 2.

Dose-response curves demonstrating the effects of high-dose-rate (panel A) and low-dose-rate (panel B) ^{60}Co γ radiation compared to 70 MeV/n proton radiation on WBCs, with blood samples taken at 24 h after irradiation. Each point represents the mean WBC count with bars representing SE. The statistical significance for the difference between the control groups and each of the irradiated groups is indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ by one-way ANOVA followed by Tukey's test). The results from two separate experiments for γ rays and three separate experiments for 70 MeV/n protons have been combined.

FIG. 3.

Exponential decay curve fitted to WBC counts in mice irradiated with γ rays and 70 MeV/n protons at the high dose rate (panel A) and low dose rate (panel B), with blood samples taken 24 h after irradiation. The dose of each radiation type required to yield 37% survival (known as the D_{37} , which equals $1/-k$) was determined and used to calculate the RBE value shown in each figure for protons compared to γ radiation [$D_{37(\gamma)}/D_{37(\text{protons})}$ or $-k_{(\text{protons})}/-k_{(\gamma)}$]. Each point represents the mean WBC count with bars representing SE. The statistical significance of the difference between the control groups and each of the irradiated groups is indicated by asterisks (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ by one-way ANOVA). The results from two separate experiments for γ rays and three separate experiments for 70 MeV/n protons have been combined.

FIG. 4.

Exponential decay curve fitted to WBC counts in mice irradiated with γ rays and 50 MeV/n protons at the high dose rate (panel A) and low dose rate (panel B), with blood samples taken 24 h after irradiation. The dose of each radiation type required to yield 37% survival (known as the D_{37} , which equals $1/-k$) was determined and used to calculate the RBE value shown in each figure for protons compared to γ radiation [$D_{37}(\gamma)/D_{37}(\text{protons})$ or $-k_{(\text{protons})}/-k_{(\gamma)}$]. Each point represents the mean WBC count with bars representing SE. The statistical significance of the difference between the control groups and each of the irradiated groups is indicated by asterisks (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ by one-way ANOVA). The results from two separate experiments for γ rays and 50 MeV/n protons have been combined.

FIG. 5.

Panel A: Variation between the two experiments is a minor influencing factor on WBC counts since the sum of squares (SS) attributable to variation between the two experiments accounted for only 0.20% of the total SS observed in the two experiments ($P = 0.006$). Panel B: Type of radiation (γ rays or protons) is not a significant influencing factor on WBC counts since the SS attributable to radiation type accounted for less than 0.01% of the total SS observed in the three experiments ($P = 0.109$). Panel C: Radiation dose rate (high or low) is a minor influencing factor on WBC counts since the SS attributable to radiation dose rate accounted for 11.19% of the total SS observed in the two experiments ($P < 0.001$). Panel D: Radiation dose (0.25, 0.5, 1 and 2 Gy) is the major influencing factor on WBC counts, as expected, since the SS attributable to radiation dose accounted for 59.63% of the total SS observed in the three experiments ($P < 0.001$).