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Effects of a granulocyte colony stimulating factor, Neulasta, in mini pigs exposed to total body proton irradiation

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Abstract

Astronauts could be exposed to solar particle event (SPE) radiation, which is comprised mostly of proton radiation. Proton radiation is also a treatment option for certain cancers. Both astronauts and clinical patients exposed to ionizing radiation are at risk for white blood cell (WBC) loss, which are the body's main defense against infection. In this report, the effect of Neulasta treatment, a granulocyte colony stimulating factor, after proton radiation exposure is discussed. Mini pigs exposed to total body proton irradiation at a dose of 2 Gy received 4 treatments of either Neulasta or saline injections. Peripheral blood cell counts and thromboelastography parameters were recorded up to 30 days post-irradiation. Neulasta significantly improved white blood cell (WBC), specifically neutrophil, loss in irradiated animals by approximately 60% three days after the first injection, compared to the saline treated irradiated animals. Blood cell counts quickly decreased after the last Neulasta injection, suggesting a transient effect on WBC stimulation. Statistically significant changes in hemostasis parameters were observed after proton radiation exposure in both the saline and Neulasta treated irradiated groups, as well internal organ complications such as pulmonary changes. In conclusion, Neulasta treatment temporarily alleviates proton radiation-induced WBC loss, but has no effect on altered hemostatic responses.

Keywords

proton radiation; minipigs; G-CSF

The Acute Radiation Syndrome (ARS) involves symptoms or signs occurring within hours to days following radiation exposure. The hematopoietic syndrome is characterized by a decline in blood cell counts, which can occur through radiation induced cell killing effects in circulating blood cells, stem cells, or progenitor cells in the bone marrow and can lead to

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bone marrow failure. The severity of the decline in blood cell counts is often used in the assessment of radiation dose, selection of therapy, and prognosis for an individual accidentally exposed to radiation or radioactive material.

The risk of occurrence of the ARS from exposure to radiation emitted from a large solar particle event (SPE) has been identified and is a concern for human space travel; the risk is expected to be particularly high during exploration class missions, such as those planned for the future by the National Aeronautics and Space Administration (NASA). Proton radiation is the major constituent (~96%) of SPE radiation. As part of the ARS, the hematopoietic syndrome resulting from doses and dose-rates expected during an SPE has been characterized, with dose-dependent toxicity to circulating leukocytes observed [1–9].

A number of countermeasures have been evaluated for effects on the ARS, which can precede radiation-induced lethality. One agent that is approved by the United States Food and Drug Administration to treat the hematopoietic syndrome is granulocyte colony stimulating factor (G-CSF), which stimulates hematopoietic cell proliferation in the bone marrow compartment. Endogenous G-CSF is a lineage specific colony-stimulating factor which is produced by monocytes, fibroblasts, and endothelial cells. There are two forms of G-CSF, which include Neupogen (filgrastim) and Neulasta (pegfilgrastim or the pegylated form of human G-CSF), a more stabilized form of filgrastim. Filgrastim is maintained in the Strategic National Stockpile as critical medical countermeasures, specifically for radiological/nuclear events affecting white blood cell counts and Pegfilgrastim is also indicated in patients (adults and children) with acute radiation injury [10]. Previously, the administration of Neupogen or Neulasta in mice immediately after exposure to gamma or proton total body radiation reduced myelosuppression, in terms of leukocyte/granulocyte counts [5]. When G-CSF was administered to dogs 2 hours after gamma radiation exposure, lethal myelosuppression was avoided, but when it was administered to dogs on day 7 after radiation exposure, all of the dogs perished [11]. Similar results using a non-human primate model, reveal that filgrastim initiated 1 day after 7.5 Gy photon radiation (the pre-determined lethal radiation dose in 50% of the population within 60 days), reduced mortality compared to the control group [12]; however, administration of filgrastim 48 hours after radiation exposure did not improve survival, compared to controls [13]. In a porcine model, G-CSF administered 1 day after gamma-irradiation resulted in a 37.5% reduction in mortality, compared to the saline treated, irradiated group [14]. From these small and large animal model studies, it is clear that the efficacy of G-CSF as a medical countermeasure to mitigate radiation-induced lethality is dependent on the time of administration.

Previously, a dose-dependent reduction in white blood cells was observed in Yucatan mini pigs, the same strain used in the current study, after exposure to proton simulated SPE radiation [15]. Radiation-induced coagulopathies/hemorrhaging have been identified in experimental animal model systems, as well as in humans after accidental radiation exposure [2, 9, 16]. We have hypothesized that such radiation induced alterations/coagulopathies can develop into disseminated intravascular coagulation (DIC) and lead to death in ferrets [9, 17, 18]. In the current study, Neulasta was administered to Yucatan minipigs to evaluate its protective effects on hematological parameters after exposure to SPE-like proton radiation. From the results of our previous studies in mice exposed to SPE-like proton radiation and

treated with Neulasta [5], Neulasta treatment in this large animal (porcine) model is expected to mitigate the effects of proton total body irradiation (TBI) on hematopoietic endpoints.

Methods

Animals

Six male Yucatan minipigs aged 12–15 weeks were purchased from Sinclair Bio Resources, LLC (Auxvasse, MO). Animals were acclimated for 7 days and were housed individually with ad lib access to water and fed standard mini-piglet chow twice daily. The animal care and treatment procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Upon acclimation, the animals were randomly assigned to 2 groups and exposed to 2 Gy of proton total body irradiation. The first group received subcutaneous injections of Neulasta (0.1 mg/kg) on days 4, 7, 10, and 13 post-irradiation (n=3). Previously, our laboratory determined that the first neutrophil nadir after 2 Gy total body irradiation in mice occurred at 4 days post-exposure, with a second nadir at 16 days post-exposure [5]. In the mouse study, multiple injections with Neulasta were effective in stimulating neutrophil release into the circulation. This is consistent with observations by Farese et al. [19] indicating that more than one dose of Neulasta was more effective than a single dose in improving the speed of hematopoietic recovery after total body irradiation in Rhesus monkeys. Although pharmacokinetic-pharmacodynamic studies were not performed here in the minipigs, it has been documented that the terminal half-life of Neulasta is approximately 15–80 hours in patients with cancer [20]. Further, in normal monkeys, Neulasta was cleared from circulation in approximately 2 days [21]. Based on this information and the given supply of Neulasta for each minipig, the authors chose to administer Neulasta every 3 days, starting on day 4 post-radiation exposure. Simultaneously, the second group of pigs were exposed to proton irradiation under the exact conditions, but received subcutaneous injections of sterile phosphate buffered saline (PBS) on days 4, 7, 10, and 13 post-irradiation (n=3).

Physics and Dosimetry

To obtain a 2 Gy homogeneous dose of proton radiation to the pigs, 37 different proton energies were used, which ranged from 103 MeV to 223 MeV at the IBA dedicated pencil beam spot scanning (PBS) nozzle exit. A 75-mm water equivalent thickness range shifter (RS) was employed immediately following the nozzle exit to degrade the proton energies to ~20 MeV to ~190 MeV, which represented proton energies characteristic of SPE radiation. The RS was necessary for more efficient transport of proton energies below 100 MeV and to increase the spot size for more robust beam delivery. The monitor unit (MU) of each proton energy was optimized to generate uniform dose from the surface to a depth of 230 mm. One MU corresponds to 3 nanoCoulombs (nC) of charge collected in a 1 cm gap air-filled ion chamber prior to the nozzle exit. Each proton energy layer was scanned in 31×21 spot patterns to cover a projected area of 580 mm (cranial-caudal) by 340 mm (ventral-dorsal) at 1200 mm from the isocenter of the PBS nozzle.

For the radiation exposures, non-anesthetized animals were restrained in an aerated plexiglass chamber large enough to provide normal postural movements. The proton beam was delivered to one side of the long axis of the pig's body, after which the entire irradiation chamber was rotated 180 degrees and an identical dose (1 Gy) was delivered to the opposite side of the pig.

Blood cell count analyses

Whole blood samples were collected from each anesthetized animal (cranial vena cava) and placed into a collection tube containing EDTA and analyzed using a Bayer Advia 120 Hematology Analyzer (using the porcine specific profile, Antech Diagnostics) within 24 hours of the blood sample collection.

Peripheral blood was collected from each animal on day 0 (prior to radiation exposure), and on days 2, 7, 10, 13, 15 (n=3, PBS treated animals only), or 16 (n=3, Neulasta treated animals only) and 30 post-radiation. Average blood cell counts were calculated for each treatment group (n=3). The pre-irradiation baseline average for each cell type represents the average from the total of 6 animals.

Coagulation/Thromboelastography analyses

At the time of whole blood collection (described above), a separate sample was placed into a collection tube containing 3.8% sodium citrate at a recommended dilution of 1:9 citrate: blood ratio. All samples were run on the rotational thromboelastogram (ROTEM, Munich, Germany) within one hour after the initial blood draw. Briefly, citrated whole blood was added to a cuvette filled with a tissue factor activation reagent and calcium chloride and analyzed on a ROTEM system at the Children's Hospital of Philadelphia, as described previously [9]. Samples were analyzed for 90 minutes. The thromboelastography parameters measured were the clot time or reaction time (R), which depicts the time from the start of a sample run until the first significant levels of detectable clot formation (2mm clot formation), clot formation time (CFT or K time) which is the time to reach a clot size from 2mm above baseline to 20mm above baseline, the alpha angle, which is a function of the rate (the kinetics) of clot development, and maximum clot firmness (MCF), which is the measurement of maximum strength or stiffness of the developed clot.

Computed tomography (CT) angiography

Animals were anesthetized by isoflurane inhalant. Animals were placed in sternal recumbency in a 16-slice Multi-Detector Computed Tomography unit (GE BrightSpeed, General Electric Company, Milwaukee, WI). Pre-contrast scans of the thorax and abdomen were acquired. A dynamic perfusion CT study of the liver was performed by scanning repeatedly an area of the liver (4 contiguous locations of 5 mm) over 60 seconds at 80 kVp after injection of a bolus of 0.5 ml/kg of iodinated contrast material (iohexol 350mgI/ml) at 4ml/sec using a power injector (GE Nemoto Dual-Shot injector, Waukesha, WI) at a maximum pressure of 2067 kP. After the perfusion study, a post-contrast scan of the whole body was obtained after injection of another bolus of 1.7 ml/kg of the same contrast material using the same injector and injection parameters. Thoracic scans were obtained at a thickness of 1.25 mm/120kVp with a lung algorithm and abdominal scans at a thickness of

2.5 mm/120kVp with a detail algorithm. A separate non-irradiated group of 3 animals (age matched to the other experimental animals evaluated by CT angiography) were subjected to the same CT protocol.

Statistical analyses

Statistical significance for the differences between the pre-irradiation baseline value and the value at each time point after irradiation was determined by one-way ANOVA followed by the Holm-Sidak test. The statistical significances at $p < 0.05$, $p < 0.01$ and $p < 0.001$ are indicated by *, ** and ***, respectively.

Significant differences between the Neulasta treated, irradiated group and saline treated, irradiated group were determined by an unpaired Student's *t*-test and the results are indicated by # ($p < 0.05$) and ## ($p < 0.01$).

Results

Total body proton radiation-induced blood cell toxicity is attenuated by Neulasta treatment

Six animals were exposed to 2 Gy total body proton radiation. Two days after radiation exposure, blood cell counts were significantly decreased. Total white blood cells (WBCs) were decreased by 50% ($p < 0.01$), neutrophil counts by 16%, lymphocyte counts by 86% ($p < 0.001$), monocyte counts by 89%, and eosinophil counts by 42%, of the pre-irradiation baseline average(s).

The first Neulasta treatment was initiated in 3 of the 6 irradiated animals on day 4 post-exposure and blood was collected on day 7 post-exposure. Total WBC counts were significantly increased in the Neulasta treated group compared to the saline treated irradiated group, which resulted in a 71% decrease in WBC count compared to the pre-irradiation baseline average. The average cell count in the Neulasta treated group was higher than the pre-irradiation average value on days 7 and 10, although not in a statistically significant manner (Fig. 1A). The saline treated group resulted in WBC toxicity with an observed reduction of 73%, 75%, 75% and 56% on days 10, 13, 15 and 30 post-irradiation, respectively, all of which were statistically significant, compared to the pre-irradiation baseline average (Fig. 1A).

For the Neulasta treated irradiated pigs, there were 3 additional Neulasta treatments administered, with the last treatment on day 13 post-exposure. The total WBC counts were increased in the Neulasta treated group, compared to the saline treated group, with statistical significance observed on days 7, 10, and 13 post-irradiation (compared to the saline treated group, Fig. 1A). The WBC counts appeared to decrease in the Neulasta treated group at the conclusion of the Neulasta administration (WBC counts were decreased by 35% of the pre-irradiation baseline average by day 30 post-exposure); however, this decrease was not statistically significant, compared to the baseline average.

Interestingly, the red blood cell (RBC) count decreased in both the saline and Neulasta treated, irradiated groups (Fig. 1B); however, only the Neulasta treated group resulted in a statistically significant difference in cell count on days 16 (by 29%, $p < 0.001$) and 30 (by

21%, $p = 0.01$) post-irradiation, compared to the RBC pre-irradiation baseline average (Fig. 1B). It is not entirely surprising that in turn, the hemoglobin and hematocrit levels were also significantly decreased at these time points in the Neulasta treated, irradiated group ($p < 0.001$, data not shown). The hemoglobin levels were not decreased in a statistically significant manner in the saline treated group throughout the 30 day experimental period, although the hematocrit level decreased to 31% ($p = 0.02$) of the pre-irradiation baseline average in the saline treated group (which was similar to the decrease observed in the Neulasta treated group, data not shown).

The neutrophil counts decreased significantly by 62%, 64%, 70% and 71% on days 7, 10, 13 and 15, respectively, after irradiation as compared to the pre-irradiation baseline average, in the saline treated group (Fig. 2). The neutrophil count in the Neulasta treated group was significantly higher than the neutrophil count in the saline treated group on days 7, 10 and 13 post-exposure, by 78%, 83%, and 74%, respectively (Fig. 2). By the 30 day time point, neutrophil counts were not decreased in a statistically significant manner in either the saline or Neulasta treated group, compared to the pre-irradiation baseline average; however, the average neutrophil count in the Neulasta treated group was 43% higher than the average count for the saline treated group.

Lymphocytes are the most radiosensitive blood cell type. The lymphocyte count decreased significantly by 87%, 81%, 82%, 80%, 77%, and 63% on day 2, 7, 10, 13, 15 and 30, respectively, after irradiation, compared to the pre-irradiation baseline average, in the saline treated group (Fig. 3A). In the irradiated animals treated with Neulasta, the lymphocyte count decreased significantly by 59%, 65%, 68%, 64%, and 62% on day 7, 10, 13, 16 and 30, respectively, after irradiation, compared to the lymphocyte count before irradiation. The lymphocyte count for the Neulasta treated group was significantly higher than the lymphocyte count for the saline treated group on days 7, 10, and 13 after irradiation, and the differences between the results in the Neulasta treated and the saline treated groups were statistically significant (Fig. 3A).

Monocytes, the other agranulocyte of the WBC types, were also significantly decreased after radiation exposure (Fig. 3B). The monocyte count decreased significantly by 87% on day 13 after irradiation, compared to the monocyte pre-irradiation baseline average. For the Neulasta treated group, the monocyte count did not decrease significantly at any time point after irradiation ($p = 0.15$), compared to the monocyte pre-irradiation baseline average. The differences between the Neulasta treated and the saline treated groups on day 13 post-radiation were statistically significant (Fig. 3B).

Lastly, platelet counts were decreased in both treatment groups, but the change was not statistically significant throughout the experimental time frame (data not shown). By day 10 post-irradiation, the average platelet count was decreased by 65% and 75% of the pre-irradiation baseline average platelet count in the saline and Neulasta treated groups, respectively and by 30 days post-irradiation, the average platelet counts remained decreased by approximately 15% (saline treated) and 75% (Neulasta treated) of the pre-irradiation baseline control value. It should be noted that the probable cause of the lack of statistical significance relates to the fact that platelet clumping was observed in all 6 irradiated animals

by day 10 post-irradiation and that the changes reported are the highest estimated platelet counts.

Clotting kinetics are altered after proton radiation exposure with and without Neulasta treatment

For both the saline and Neulasta treated groups, the R did not change when compared to the pre-irradiation baseline average (Table 1). All other parameters were changed by day 10 post-irradiation in both the saline and Neulasta treated groups (Table 1). The CFT increased significantly by day 13 post-exposure, but returned to near baseline average CFT by day 30 post-irradiation. The alpha angle, the slope between R and CFT, which is more comprehensive than just CFT alone, was significantly reduced by day 10 post-exposure, and remained significantly reduced throughout the 30 day study (with the exception of the Neulasta treated group on day 30 post-exposure which exhibited a decreased alpha angle, compared to the pre-irradiation baseline average, but this decrease was not statistically significant). The results from the MCF measurements followed the same trend as the alpha angle measurement results, with statistical significance starting at day 10 post-exposure. It was determined that the differences between the results for the Neulasta and saline treated groups were not statistically significant using an unpaired Student's t-test (data not shown).

CT angiography report

Thorax and abdominal angiography was performed approximately 2–3 weeks after radiation exposure and the findings were similar in the saline and Neulasta treated groups. The findings are listed in Table 2 with potential implications recognized as possible radiation pneumonitis, thromboembolic events, or atelectasis. All 6 irradiated animals (3 saline treated and 3 Neulasta treated) presented with lung abnormalities. It is important to note that 2/3 non-irradiated, age-matched animals also presented with mild patchy opacities in the lung field, indicating that this observation (also reported in the irradiated animals) may not be a lung abnormality for this species at all. However, without the availability of results from lung biopsy data, the potential diagnoses of potential thromboembolic events in the irradiated animals cannot be ruled out. Examples of lung abnormalities (reported in Table 2) are shown in Figure 4. The only other abnormality observed in the irradiated animals was a small cortical hypoattenuating area in the cortex of the left kidney in one animal (irradiated, treated with Neulasta, ID 25033), which may represent small hematomas in the kidney. As mentioned above, 2/3 non-irradiated animals presented with lung abnormalities (no other abnormalities noted) and the third non-irradiated animal did not present with any abnormalities upon CT scanning.

Discussion

Here, we provide evidence that SPE-like proton radiation results in hemostatic changes, both with and without Neulasta treatment, which could be observed as early as 10 days post-irradiation. SPE-like proton radiation also resulted in dose-dependent toxicity to hematopoietic cells, and the different cell types evaluated did not return to their pre-irradiation, baseline levels during the 30 day study. In the irradiated pigs, Neulasta treatment resulted in transient improvements in white blood cell counts, primarily due to increases in

granulocyte counts, but it did not affect the observed hemostatic changes. Lastly, CT angiography evaluations suggested that radiation exposure resulted in possible thromboembolic changes observed as early as 2 weeks post-irradiation.

The dose of proton radiation used in this study is considered to be of conventional radiation therapy fraction size (1.8 – 2.0 Gy), with typical total doses >50 Gy, depending on the cancer type. In terms of astronaut health, a large solar particle event (SPE), consisting mostly of protons, may result in doses to the blood forming organs during an extravehicular activity as high as 1.4 Gy (estimated from event spectra of a historical large SPE) [22]. Further, a hypothetical worst-case SPE spectrum estimates radiation doses to the bone marrow from 1.09 – 2.81 Gy, assuming 10 – 1 g cm⁻² of aluminum shielding (areal density), respectively [23, 24], which coincides with the proton dose (2 Gy) used in the current study.

The inflammatory, immune, and hemostatic systems work in conjunction to produce a coordinated response to injury. For example, WBCs are recruited to sites of vascular and tissue injury and are partly responsible for activating an inflammatory response that may activate or inhibit thrombosis. Exposure to proton radiation resulted in significantly decreased WBC counts that did not recover to baseline levels by 30 days post-irradiation. Neulasta treatment resulted in a significant increase in total WBC and neutrophil counts; however, the Neulasta-stimulated blood cell count increase was only observed up to about day 15 post-radiation (of the 30 day observation period), which more than likely corresponds to the last Neulasta treatment, administered on day 13 post-radiation. It is not surprising that the blood cell counts appeared to decline after the last Neulasta administration, since the reported half-life ranges from 15 to 80 hours after injection [25]. These results align with mouse studies demonstrating that the neutrophil counts of mice treated with Neulasta after a 2 Gy gamma radiation exposure increased in a statistically significant manner and remained increased for a period of 2 days after the last Neulasta treatment [5]. Neupogen treatment in irradiated mice lasted for only approximately 1 day after drug administration [5]. Similarly, Moroni et al. [14] recently showed a similar trend in increased WBC and neutrophil counts in mini pigs exposed to 1.78 Gy gamma-ray irradiation and G-CSF treatment, which was only observed on the days of drug administration and approximately 1 day after the last drug administration. These data suggest that both Neupogen and Neulasta treatment result in transient increases in hematopoietic cells after radiation exposure. This is the first report on the effects of Neulasta on proton radiation-induced hematopoietic cell toxicity, at an intermediate dose of 2 Gy, in a large animal model.

Contrast-enhanced computed tomography changes consistent with possible pneumonitis, thromboembolic events, and/or atelectasis manifested in 6/6 irradiated animals in this study as early as 2–3 weeks post-irradiation. 4/6 of the irradiated animals and 0/3 of the non-irradiated animals presented with lesions suggesting possible thromboembolism. Interestingly, thrombocytopenia was observed in all 6 irradiated animals. Platelet clumping was also observed, which suggests an activation of the clotting cascade that would further compromise hemostasis. Nevertheless, the radiation-induced altered TEG parameters observed as early as 10 days post-irradiation can be considered a predictive risk for the radiation-induced thrombosis observed in some of these animals. Taken together, these

results provide some evidence that radiation-induced changes capable of leading to thromboembolism can occur at a high frequency and within 2 weeks post-irradiation exposure. These results suggest that further investigation (with larger numbers of animals evaluated) on this phenomenon is warranted. Overall, Neulasta treatment did not affect the observed radiation-induced alterations in TEG parameters, which is consistent with the multi-systemic hemorrhaging in gamma-irradiated pigs after treatment with either saline or G-CSF treatment, as reported by Moroni et al. [14].

An additional finding from this study was the potential incidence of suspected radiation pneumonitis in the irradiated animals (both saline and Neulasta treated groups). 4/6 irradiated animals presented with alveolar pattern changes (n=3) and/or ground glass opacities (n=3) on CT images, although patchy ground-glass interstitial opacities were also observed in 2/3 non-irradiated animals. These data suggest that these patchy ground glass opacities may represent a normal CT finding in this species. Although the alveolar lesions could be due to radiation pneumonitis, other possibilities include atelectasis associated with anesthesia (since they were mostly ventral) or pneumonia. The lung changes were observed 2–3 weeks after proton radiation exposure, with no apparent clinical symptoms observed, such as cough, dyspnea, or fever. In clinical trials focusing on radiation pneumonitis (as a result of targeted lung or breast radiotherapy), it is recorded that the onset of pneumonitis typically occurs between 1–3 months after the completion of therapy, involving 1.0 – 2.5 Gy fractions, for total prescribed doses ranging from 25 – 74 Gy [26, 27]. It is estimated that 20–30% of patients undergoing targeted lung radiotherapy will develop acute pneumonitis and 2% of these patients have a risk of fatal pneumonitis [28]. By the conclusion of the current 30 day experiment, the animals were still not presenting with symptoms of pneumonitis, but it was not determined whether the alveolar changes observed on the thorax CT resolved on their own in the affected animals. It is noteworthy that radiation pneumonitis or radiation-induced pneumonopathy was observed in our previous studies utilizing Yucatan mini pigs exposed to various doses of electron radiation designed to deliver an SPE-like dose distribution in irradiated animals, with higher external and lower internal doses [7].

It was observed that Neulasta treatment did not alter the radiation-induced hemostatic responses, as the thrombotic parameters were not different in the Neulasta-treated pigs compared to those observed in the irradiated pigs treated with saline. The proton radiation-induced changes in hematology and hemostasis observed in this study may impact crew member health, performance, and mission completion. Further, the effect of proton radiation on all the parameters reported here may be amplified by the additive/synergistic effects of microgravity, as has been observed in numerous previous studies [29–31]. Therefore, the pursuit of suitable countermeasures, or combination of countermeasures, is imperative for crew and patient safety. From the results reported here, the Neulasta stimulated increase in the numbers of circulating WBCs appears to be temporary, but it can be an effective countermeasure for WBC loss resulting from exposure to SPE-like proton radiation.

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A.

B.

Figure 1.

Total WBC counts (A) are decreased in animals exposed to proton TBI, with results attenuated by Neulasta treatment. RBCs (B) are decreased in irradiated animals treated with

Neulasta. Animals were exposed to 2 Gy proton TBI (n=6, day 0) and on day 4 post-irradiation treatment with either saline (black circles) or Neulasta (white circles) commenced (n=3 for each group at each time point evaluated, except for the day 0 for time point, at which n=6). The RBC count for the irradiated animals treated with Neulasta was not significantly different from the RBC count for the irradiated animals treated with PBS at any time points after irradiation ($p = 0.13$). Values are expressed as the average $\pm$ SEM.

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Figure 2.

Neutrophil counts are decreased in animals exposed to proton TBI by day 7 post-irradiation, with results attenuated by Neulasta treatment. Animals were exposed to 2 Gy proton TBI (n=6, day 0) and on day 4 post-irradiation, treatment with either saline (black circles) or Neulasta (white circles) commenced (n=3 for each group at each time point evaluated, except for the day 0 time point, at which – n=6. Values are expressed as the average $\pm$ SEM.

A.

B.

Figure 3.

Agranulocytes (lymphocytes [A] and monocytes [B]) are decreased in animals exposed to proton TBI by day 1 post-irradiation, with results attenuated by Neulasta treatment. Animals were exposed to 2 Gy proton TBI (n=6, day 0) and on day 4 post-irradiation treatment with either saline (black circles) or Neulasta (white circles) commenced (n=3 for each group at each time point evaluated, except for day 0 time point, at which – n=6).

Figure 4.

CT image of lungs of proton irradiated animals, with and without Neulasta treatment. The lungs of a saline-treated, irradiated animal (ID 25044) display a patchy central enhancement at arterial phase (indicated by arrows) at 3 weeks after proton total body irradiation exposure (A). Considerations reported by a certified radiologist (Dr. W. Mai) include pneumonitis +/- transient vasoconstriction vs thromboembolism. The lungs of a Neulasta-treated, irradiated animal (ID 25023) presented with a subpleural wedge-shaped lesion in the dorsal aspect of the right caudal lung lobe (B, left panel, arrow). In the left caudal lung lobe, one example of a small nodule is shown on the right panel (arrow, several nodules in the deeper lung parenchyma were also noted). Considerations for these findings in this animal include thromboembolism, embolic pneumonia, radiation pneumonitis, scarring from previous disease or granuloma of other origin. The images of the left and right lungs shown here were obtained at 2 weeks post-irradiation.

Table 1

Thromboelastography analysis

Parameter	Treatment	ANOVA	Pre- irradiation	1 day post	7 day post	10 day post	13 day post	30 day post
R	Saline	ns	57.0 ± 4.3	55.7 ± 4.9	62.7 ± 2.9	56.3 ± 7.0	57.0 ± 5.3	84.3 ± 42.3
	Neulasta	ns	59.7 ± 9.9	58.0 ± 11.3	71.3 ± 10.0	75.3 ± 2.5	72.3 ± 3.5	51.3 ± 31.0
CFT	Saline	p < 0.05	48.9 ± 5.5	54.7 ± 6.4	70.3 ± 13.6	142.7 ± 22.6	739.0 ± 551.8*	167.8 ± 70.8
	Neulasta	ns	48.0 ± 3.6	52.0 ± 2.0	69.0 ± 2.6	161.7 ± 36.7	629.0 ± 804.5	108.0 ± 41.1
Alpha angle	Saline	p < 0.001	81.3 ± 0.6	80.3 ± 1.2	77.0 ± 2.6	64.3 ± 3.2*	54.3 ± 5.5*	61.7 ± 12.1*
	Neulasta	p < 0.01	81.7 ± 0.6	80.7 ± 0.6	78.0 ± 1.0	64.7 ± 1.2*	61.3 ± 10.8*	70.7 ± 7.6
MCF	Saline	p < 0.001	77.7 ± 0.6	80.3 ± 7.6	72.7 ± 3.8	55.3 ± 5.5*	29.7 ± 12.5*	51.0 ± 12.1*
	Neulasta	p < 0.001	79.7 ± 0.6	76.7 ± 0.6	78.7 ± 6.7	49.7 ± 7.5*	33.0 ± 11.5*	67.0 ± 14.1

R, reaction time; CFT, clot formation time; MCF, maximum clot firmness. Values are expressed as the average $\pm$ SD (n=3). Statistical significance for TEG parameters was evaluated using a repeated one-way ANOVA followed by a post-Tukey test.

Table 2

Findings on CT Angiography

Treatment	CT report	Conclusions
2 Gy + Saline (ID 25028)	1 Mild patchy opacities in the peripheral lung field with a ground glass appearance, almost alveolar in some areas such as medial aspect of the right caudal lung lobe*	1 Possible radiation pneumonitis vs atelectasis vs thromboembolic event
2 Gy + Saline (ID 25032)	1 Mild multifocal patchy interstitial disease with a ground glass appearance* 2 Focal patchy alveolar disease right and left cranioventral lung fields	1 Possible radiation pneumonitis vs atelectasis vs thromboembolic event 2 Pneumonia vs atelectasis associated with anesthesia
2 Gy + Saline (ID 25044)	1 Heterogeneous pulmonary enhancement at arterial phase in central pulmonary territories (mosaic pattern) with comparatively lack of enhancement of peripheral lung tissue at arterial phase	1 Pneumonitis +/- transient vasoconstriction vs thromboembolism
2 Gy + Neulasta (ID 25023)	1 Ventral multifocal alveolar pattern 2 Patchy peripheral ground glass opacities* 3 Wedge shaped sub pleural opacities 4 Few small pulmonary nodules in deeper lung field	1 Atelectasis associated with anesthesia vs pneumonia 2 Pneumonitis 3 Thromboembolism embolic pneumonia, scarring from previous disease 4 Radiation pneumonitis, embolic pneumonia, granuloma of other origin
2 Gy + Neulasta (ID 25033)	1 Small cortical hypoattenuating areas in the cortex of the left kidney 2 Heterogeneous pulmonary enhancement at arterial phase in central pulmonary territories (mosaic pattern) with comparatively lack of enhancement of peripheral lung tissue 3 Multifocal ventral alveolar lung pattern phase*	1 Infarcts vs small hematomas 2 Pneumonitis +/- transient vasoconstriction vs thromboembolism 3 Atelectasis secondary to anesthesia vs pneumonia
2 Gy + Neulasta (ID 25035)	1 Ventral multifocal alveolar pattern* 2 In the right mid to ventral caudal lung lobe, there are several wedge shaped areas of subpleural infiltrate	1 Atelectasis vs thromboembolic event
0 Gy (ID 25029)	1 Mild hazy patchy multifocal interstitial (ground glass) areas*	1 Possible normal variation in this species
0 Gy (ID 25031)	1 No abnormalities noted	1 No abnormalities noted
0 Gy (ID 25053)	1 Mild diffuse interstitial pattern in the lung*	1 Atelectasis and/or normal variation in this species

* NOTE: the mild peripheral patchy ground glass pattern was also observed in 2/3 non-irradiated pigs and therefore this may be a normal variation in pigs.

All results and potential conclusions were reported by board-certified veterinary radiologists. d-certified veterinary radiologists.