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Acute Biological Effects of Simulating the Whole-Body Radiation Dose Distribution from a Solar Particle Event Using a Porcine Model

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Abstract

In a solar particle event (SPE), an unshielded astronaut would receive proton radiation with an energy profile that produces a highly inhomogeneous dose distribution (skin receiving a greater dose than internal organs). The novel concept of using megavoltage electron-beam radiation to more accurately reproduce both the total dose and the dose distribution of SPE protons and make meaningful RBE comparisons between protons and conventional radiation has been described previously. Here, Yucatan minipigs were used to determine the effects of a superficial, SPE-like proton dose distribution using megavoltage electrons. In these experiments, dose-dependent increases in skin pigmentation, ulceration, keratinocyte necrosis and pigment incontinence were observed. Five of 18 animals (one each exposed to 7.5 Gy and 12.5 Gy radiation and three exposed to 25 Gy radiation) developed symptomatic, radiation-associated pneumonopathy approximately 90 days postirradiation. The three animals from the highest dose group showed evidence of mycoplasmal pneumonia along with radiation pneumonitis. Moreover, delayed-type hypersensitivity was found to be altered, suggesting that superficial irradiation of the skin with ionizing radiation might cause immune dysfunction or dysregulation. In conclusion, using total doses, patterns of dose distribution, and dose rates that are compatible with potential astronaut exposure to SPE radiation, animals experienced significant toxicities that were qualitatively different from toxicities previously reported in pigs for homogeneously delivered radiation at similar doses.

INTRODUCTION

On missions outside of the Earth's magnetosphere, astronauts may receive significant whole-body proton radiation exposures during a solar particle event (SPE). Although large SPEs are thought to occur rarely, their timing and energy spectrum cannot currently be forecasted with sufficient accuracy to completely prevent exposure of astronauts to SPE radiation. On Earth, humans are protected from this radiation by the Earth's large magnetic field. For astronauts, the spacecraft and spacesuit provide modest protection from radiation associated with these events, but that protection is further diminished during extravehicular activity (EVA). With the desire to further increase the exploration of the moon and other planets as well as with the growing interest in space tourism, it is important to evaluate the long- and short-term effects of SPE radiation.

In an SPE exposure, the whole body of an unshielded astronaut would receive radiation consisting mainly of protons with energies less than or equal to 50 MeV. This unique energy profile is predicted to produce a highly inhomogeneous dose distribution (with skin receiving a greater dose than internal organs) for which the dose-toxicity relationship is incompletely understood (1, 2). One major problem with applying traditional radiobiological paradigms to SPE radiation is that the inhomogeneous dose distribution for an SPE makes the relatively homogeneous dose distribution of the ^{60}Co γ -ray standard a less than ideal standard for RBE determinations. Moreover, the inhomogeneity of SPE dose distributions makes the potential biological effects of exposure of astronauts to SPE radiation difficult to predict using organ exposure limits derived from homogeneous radiation sources such as ^{60}Co γ rays. The novel concept of using megavoltage electron-beam radiation to more accurately reproduce both the total dose and the dose distribution of SPE protons and to make meaningful RBE comparisons between protons and conventional radiation has been described previously (3). In this study, the biological effects of an SPE-like superficial radiation dose distribution have been modeled using Yucatan minipigs exposed to electrons from a linear accelerator.

Previous SPEs (August 1972, October 1989 and September 1989) were predicted to be capable of delivering a skin dose of 32.15 Gy, 25.99 Gy and 7.68 Gy, respectively (1). It has been predicted that skin doses up to 15 Gy could lead to erythema, epilation (loss of hair), and moist desquamation (thinning of the skin with weeping due to loss of epithelial integrity) of the skin (2). Skin ulceration has also been observed in individuals exposed to radiation at doses of 2 to 20 Gy, but it has been assumed that the ulceration was due to injury to the hemopoietic system and not to the direct effect on the skin (4). The major goal of this study was to use Yucatan minipigs to characterize the cutaneous toxicity dose response for SPE-like radiation affecting predominantly the skin. An important secondary goal of these studies was to determine the impact of relatively superficial irradiation on internal organ function and to begin determining whether previous exposure limits derived from relatively homogeneous human irradiation will be useful in predicting the effects of SPE radiation on astronauts. The Yucatan minipig was chosen for this study because the pig model has been determined to be the best model of human skin due to the similarities in the structure and thickness of the skin layers. Other species such as rodents and nonhuman primates have also been considered, but due to the marked anatomical differences in rodents and the difference in thickness of the skin layers in nonhuman primates, pigs have become the model of choice (5–7). Research has also shown that pigs respond to radiation in a manner that more closely mimics the human response than rodents (5).

METHODS

Humane Care and Use of Animals

All animals were handled and all activities involved in this study were conducted under a protocol approved by the University of Pennsylvania Institutional Animal Care and Use Committee. The animals were housed in facilities that were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) and were inspected regularly by the U.S. Department of Agriculture (USDA).

CT-Based Simulation of Absorbed Dose in Pigs

Three Yucatan minipigs of the same approximate age and weight as the animals used for the remainder of this study were sedated using ketamine (Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (Lloyd Laboratories, Shenandoah, IA) (2 mg/kg and 20 mg/kg, respectively) and helical, 3-mm slice CT scans were obtained of the entire animal. Computational modeling of the dose distributions was performed using techniques adapted from radiation therapy planning for human patients. The CT scan was imported into clinical treatment planning software (Eclipse™, Varian Medical Systems, Palo Alto, CA), and the predicted dose distribution to the total body was determined using the Monte Carlo electron dose calculation algorithm (Varian Medical Systems). These simulations were performed with corrections that incorporate the inhomogeneity of the irradiated tissues into the final dose map. Using the organ contouring function of Eclipse™, the skin, lungs, pleura, spinal cord and marrow-containing bones (pelvis, vertebral bodies, shoulder girdle, humerus and femur) were outlined and volumetric doses were computed. Note that the absorbed dose for this CT is negligible (~0.1 cGy) compared to the doses being tested as simulating SPE radiation skin exposures.

Electron-Beam Exposure Setup and QA

The setup for electron-beam irradiation has been described previously (3). Briefly, a Clinac iX linear accelerator (LINAC) (Varian Medical Systems) was used to deliver 6 MeV electrons at a source-to-skin distance of 5 m. The desired dose rate was achieved by modulating the output of the LINAC to deliver the desired dose over 3 h. The entire set of electron fields produced in this study was measured using an IBA Dosimetry PPC40 parallel-plate ionization chamber and PTW electrometer. The PPC40 response was calibrated in a 6 MeV LINAC beam with an SSD of 100 cm and 1.4 cm buildup and using a 10 cm × 10-cm electron cone. In this configuration, the LINAC was calibrated to output 1 cGy/MU.

Irradiation of Animals

Three 3–4-month-old (9–10 kg) Yucatan minipigs (Sinclair Bio Resources, Colombia, MO) per group (all light gray in color except one pig that was white in color with light gray spots) were placed individually in a clear cast acrylic (Plexiglas) irradiation chamber and exposed to a total skin dose of 5, 7.5, 10, 12.5, 15 or 25 Gy of 6 MeV electrons delivered by the linear accelerator described above. Another group of three pigs was sham-irradiated and served as controls for the irradiated pigs in this study. The animals were not anesthetized for the radiation exposure and were able to express normal postural movements throughout. The entire dose was delivered over approximately 3 h, resulting in a dose rate of 1.7, 2.5, 3.3, 4.2, 5 or 8.3 Gy/h, respectively. The radiation dose was delivered in 1.25–6.25-Gy increments (one quarter of each total dose) 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 was delivered to the opposite side of the pig until the final dose was achieved. The dose, dose rates, sample size and procedural timeline are outlined in Table 1. Surface patient dosimetry

verification devices (OneDose™, Sixel Technologies, Morrisville, NC) were placed in various locations on each pig's body to provide a measurement of the amount of radiation received at each location.

Skin Monitoring for Acute Radiation Toxicity

After irradiation, the pigs were observed and evaluated using a modified version of the Common Terminology Criteria for Adverse Events (CTCAE, v 3.0) (Table 1). Animals were scored once daily for 30 days for skin pigmentation using color-coded cards and were given a pigmentation score from lightest (1=white) to darkest (8=black). A 3-mm skin biopsy was taken prior to irradiation and at days=7, 14 and 30 postirradiation. All skin biopsies were obtained from the lateral thorax and were fixed in 10% neutral buffered formalin for at least 24 h, embedded, sectioned and stained with hematoxylin and eosin (H&E). H&E-stained sections were evaluated under the direction of a board-certified dermatopathologist for radiation-induced abnormalities, including necrotic keratinocytes.

Hematopoietic and Immunological Testing

Blood was collected via the cranial vena cava under isoflurane anesthesia prior to irradiation and at 4 h, 24 h, 7 days, 14 days and 30 days postirradiation for complete blood count analysis by Antech Diagnostics (Lake Success, NY). Thirty days after irradiation, animals were euthanized and tissues were collected for histopathological analysis, with the exception of one animal that was irradiated with 12.5 Gy that was not euthanized until 9 months after irradiation. To test the effects of superficial irradiation on immunological function, serial phytohemagglutinin (PHA) skin reactivity testing was performed. Two days prior to irradiation and then on days 5, 12 and 28 after irradiation, intradermal injections of PHA (Sigma-Aldrich, St. Louis, MO) (20 µg in 50 µl of PBS) and PBS control (50 µl) were given on the lateral flank of each animal. Delayed-type hypersensitivity (DTH) responses were measured 2 days later using thickness gauge calipers, and full-thickness skin biopsies were obtained at the center of each DTH response area at 0, 7, 14 and 30 days postirradiation.

Diagnostics and Therapeutics

Under ketamine/xylazine sedation (as above), select animals with respiratory pathology clinical signs had chest radiographs obtained and a CT scan (as described above) performed. The one animal that had received 12.5 Gy and was showing respiratory issues was placed on oxytetracycline (7 mg/kg, intramuscular injection, daily for 4 days total; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) approximately 90 days postirradiation. Prednisone (0.25mg/kg, oral administration, once a day for 2 days total; Westward Pharmaceutical Company, Eatontown, NJ) was also given to the same animal approximately 120 days postirradiation.

At necropsy, serum and sterile lung samples were sent to the University of Minnesota Veterinary Diagnostic Laboratory (St. Paul, MN) for infectious disease testing, including *Mycoplasma hyopneumoniae* PCR, porcine reproductive and respiratory syndrome virus PCR, swine influenza virus PCR and *Actinobacillus pleuropneumoniae* ELISA.

Statistical Analysis

Radiation-induced changes in blood cell counts over time were analyzed and compared by one-way ANOVA. Each time was compared to the preirradiation time. Differences were considered significant at $P < 0.05$. Data for the DTH reactions were analyzed using Student's *t* test, comparing each dose group to the sham-irradiated group. The analyses and generation of graphs were performed using GraphPad Prism Software (version 5).

RESULTS

Dosimetry for Irradiated Animals

In a human exposure to SPE protons, the typical dose distribution is relatively inhomogeneous, with the surface receiving a dose 5–10 times higher than the internal organs (1). It has been shown previously that this dose distribution is markedly different from that of ^{60}Co γ radiation; 6 MeV electrons better simulate the skin dose distribution from SPE radiation (3). For this study, the dose to skin and internal organs was computed using standard CT-based treatment planning techniques with a Monte Carlo algorithm using EclipseTM treatment planning software. Organ doses determined using these methods reveal that the mean skin dose was 82.4% of the designated cohort dose, with the face, ears, dorsum and lateral sides of the pig receiving the highest doses of radiation. For the 12.5-Gy dose cohort, this corresponds to a mean dose of 10.3 Gy with a volume receiving at least 5 or 10 Gy (V5Gy, V10Gy) of 95.1% and 53.9%, respectively (Fig. 1). As expected, internal organs such as the spinal cord, lungs and marrow-containing bones received relatively low doses of radiation. It should be noted, however, that while the surface of organs such as the lungs received a higher dose than the inside, the predicted median and mean lung doses were 11% and 20.5% of the designated cohort dose, respectively. For the 12.5-Gy dose cohort, this corresponds to a V5Gy and V10Gy of 21.4% and 0.5%, respectively.

Skin Complications from Simulated SPE Irradiation Using Electrons

Six groups of three pigs each were irradiated with doses ranging from 5 to 25 Gy, delivered over 3 h, and skin was observed for clinical signs of acute radiation toxicity for 30 days postirradiation. Animals developed generalized hyperpigmentation within 5 to 10 days after irradiation that became more pronounced by 14 days postirradiation and increased with escalating dose (Fig. 2B). The most dramatic areas of hyperpigmentation were found in areas that were exposed to the highest dose of radiation (as determined by the surface dosimetry verification devices), including the face, ears, legs, dorsum and lateral sides (data not shown). Serial biopsies were performed to evaluate the skin for histopathological changes after irradiation. Figure 2A compares biopsies from an unirradiated animal (left) to those from an animal exposed to 12.5 Gy electrons (right) at 7 days. The increased deposition of melanin in the basal layer of the epidermis (black arrows) in the irradiated animal correlates with the clinically observed hyperpigmentation.

All three pigs exposed to 25 Gy were observed to have blister formation around 19 days postirradiation. These pigs also developed skin wounds or ulcerations in additional locations, including the tail, ears and legs. In the pigs that received 25 Gy, Grade 1 alopecia was observed along the dorsum that was apparent by approximately 7 days postirradiation. Over the course of the 30-day experiment, sham-irradiated control pigs did not show any change in skin color, alopecia, blistering or skin wounds and also did not have any histopathological abnormalities. Both irradiated and sham-irradiated animals appeared to have dry skin, so this was not considered to be a complication associated with exposure to radiation.

The biopsy sites were monitored closely to determine whether animals experienced radiation-impaired wound healing. At 25 Gy, 6% of biopsy sites (3 out of 48 total) showed failure to heal with clinically evident skin necrosis (designated Grade 5, Fig. 2C). The remaining sites as well as all other biopsy sites on animals that received lower doses of radiation healed without difficulty. All skin biopsies were evaluated histologically by a board-certified dermatopathologist. Necrotic keratinocytes and pigment incontinence were observed upon histopathological evaluation (Fig. 3).

Pigment incontinence is a term that describes the presence of melanin pigment in the dermis, either as free pigment granules or in melanophages. This happens when there is an insult to the epidermis that impairs pigment transfer from melanocytes to keratinocytes. It can also happen when pigment containing cells undergo necrosis. If radiation promotes these cellular events, then the presence of melanophages would be reasonable.

These effects appeared to be dependent on dose, and of the days the biopsies were taken, the most pronounced effect was observed at 14 days postirradiation, with almost complete resolution by 30 days postirradiation. Twelve days after exposure to a 12.5-Gy total skin dose, one pig was found to have blisters and multiple small ulcerations on the dorsum that may have been due to overlap of fields from positional shifting of the animal during irradiation. This animal was not euthanized until 9 months after irradiation to allow time to observe the healing response of the skin, and it was found to have healed completely 30 days postirradiation with no sequelae (grossly or histologically) specific to these ulcerated areas within the 9-month period.

Internal Organ Complications from Simulated SPE Irradiation Using Electrons

Five animals (one each exposed to 7.5 Gy and 12.5 Gy and three exposed to 25 Gy) developed symptomatic, radiation-associated pneumonopathy that radiographically involved all lung fields but was worse in the pleura and apices, where the radiation dose was found to be highest (estimated from the Eclipse™ software program). One of three animals receiving 7.5 Gy and one of three animals receiving 12.5 Gy developed nonproductive coughs 1 day and 93 days postirradiation, respectively. Thoracic radiographs were obtained on the pig that had received 12.5 Gy, and atelectasis of the right cranial lung lobe was present. A diagnostic CT scan was also performed, and alveolar consolidation along with areas of ground glass attenuation (hazy lung opacity), with mild mid to peripheral lung bronchial wall thickening, and mild peripheral bronchiectasis were found (Fig. 4A). Atelectasis of the right cranial lung lobe, as noted on the radiographs, was persistent. The CT findings were consistent with an acute lung injury (ground glass attenuation and alveolar consolidation) concurrent with chronic bronchial changes and volume loss. Differential diagnoses for these findings were radiation-induced lung injury or atypical infectious bronchopneumonia. To help differentiate between these possibilities, sequential antibiotic therapy that included atypical (includes mycoplasma), gram-positive and gram-negative coverage was initiated. There was no clinical response after 2 weeks of antibiotic therapy. Corticosteroid therapy was then instituted, and symptoms improved rapidly with dramatic improvement and resolution of imaging abnormalities within 1 month. A clinical diagnosis of radiation-associated pneumonopathy was made based on these findings.

Three animals that received 25 Gy developed a similar radiographic and clinical pneumonopathy, observed between 5 days and 19 days after irradiation. Histopathology was performed after the animals were euthanized at 30 days. A peribronchial increase in bronchus-associated lymphoid tissue, septal edema, mononuclear infiltrate, pleural thickening, subpleural fibrosis and broncho-interstitial pneumonia were found (Fig. 4B). In addition, bronchial samples from all three pigs tested positive for *Mycoplasma hyopneumoniae*, confirmed by polymerase chain reaction (PCR). Thus, at the highest radiation dose, a complex pneumonopathy developed with a mycoplasmal pneumonia superimposed on an acute radiation pneumonitis that were prominent both pathologically and radiographically the areas of the lung receiving the highest dose (as estimated by the Eclipse™ software program).

In addition to the respiratory signs, one of the three animals exposed to 25 Gy became slightly lethargic and developed decreased lower extremity strength with ataxia and hyperreflexia beginning 7 days postirradiation. These observations are clinically consistent

with an acute spinal radiation myelopathy. No gross abnormalities to the central nervous system were detected upon necropsy at 30 days. Histopathology of the one animal exhibiting clinical signs revealed that the cerebellum was extensively rarefied (characterized by increased vacuolization in the gray matter) with no inflammation or vascular changes noted (data not shown).

White Blood Cell Effects from Simulated SPE Irradiation

The results indicate a statistically significant reduction in the WBC count 1 day after the 25-Gy dose of radiation that recovered by 7 days and increased significantly above the preirradiation level by 30 days postirradiation compared to the preirradiation values (Fig. 5A). There were no statistically significant changes in the overall average WBC count for the other dose groups.

Decreases in the average lymphocyte count were observed in the 15-Gy (Fig. 5B) and 25-Gy (Fig. 5C) dose groups. In the 15-Gy dose group, the average lymphocyte count was decreased in a statistically significant manner 4 h (approximately 63% of the preirradiated count), 1 day (approximately 42% of the preirradiated count), and 7 days (approximately 65% of the preirradiated count) after radiation exposure (Fig. 5B). In the 25-Gy dose group, a similar trend in lymphocyte reduction was observed as in the 15-Gy dose group; however, only the 1-day point was statistically significant compared to the preirradiation values (Fig. 5C), with an average lymphocyte count of only 29% of the baseline, preirradiation cell count. The absolute neutrophil count was increased at 30 days but only for the 25-Gy dose group (Fig. 5D). No other dose groups showed any statistically significant changes in lymphocyte and neutrophil counts.

Immunological Changes

The quantitative changes in WBC do not appear to be sufficient to explain the radiation-induced pneumonitis and mycoplasmal pneumonia in the 25-Gy animals. These findings suggest that superficial irradiation with 6 MeV electrons could lead to immunological dysfunction, leaving the body more susceptible to infection and inflammation-mediated organ damage. To evaluate the effects of superficial irradiation on immunological function, specifically the DTH response, serial PHA skin reactivity testing was performed. The results of these experiments indicate that superficial irradiation leads to an increased level of delayed type hypersensitivity over the 30-day experiment, reaching maximum levels at 7 days (Fig. 6), suggesting that this inhomogeneous pattern of dose deposition might lead to immunological dysfunction. The DTH response was statistically significant for all doses (excluding 10 Gy) compared to the sham-irradiated animals at 7 days (Fig. 6). A similar trend was observed at 14 and 30 days (data not shown).

DISCUSSION

Electron radiation was used to simulate the total doses, patterns of dose distribution and dose rates for an SPE. In the skin, exposure to simulated SPE radiation using 6 MeV electrons at doses up to 25 Gy causes hyperpigmentation, excessive desquamation and histopathological changes in pigmented skin. The hyperpigmentation observed was shown histologically to be the result of increased melanin deposition in the epidermis. Previous studies have also shown increased pigmentation to be connected to dermal or epidermal deposition of melanin, whereas sham-irradiated, pigmented pigs do not demonstrate melanin in the dermis (5). The time postirradiation until the appearance of hyperpigmentation did not appear to be affected by dose in previous studies (5) or in this study. A range of times until the onset of clinical signs in the skin has been reported, suggesting that the time to onset is not related to dose and radiation quality (5–10). Approximately 10% of humans exposed to a skin dose of

22–52 Gy of megavoltage radiotherapy experience erythema, tanning (or hyperpigmentation) and desquamation (11). Due to the use of light gray pigs in this study, it was difficult to assess the degree of erythema that may have been present after irradiation. Other studies that have used both pigmented and unpigmented pigs have also found that erythema could not be evaluated in the pigmented pigs, although sufficiently high doses of radiation caused erythema to the skin of white pigs (5). The histopathological changes (pigment incontinence and keratinocyte necrosis) observed in the biopsies have also been observed in human patients who experienced radiation dermatitis after fluoroscopic radiation overdose (12).

The skin toxicity observations reported here are consistent with previous studies describing the acute response of human and porcine skin to these doses. For example, humans exposed to fluoroscopy at doses greater than 2 Gy have developed erythema and epilation of the skin, with desquamation also observed at skin doses between 10 and 15 Gy (13). Irradiating pigs with a single dose of 250 kV X rays resulted in an ED₅₀ for early moist desquamation of approximately 27.26 Gy (6). In this study, it also appears that doses greater than 20 Gy may cause a loss of skin integrity, resulting in blistering and ulceration. These lesions may be painful and could become infected, which could be detrimental to an astronaut during a mission. It has been found that astronauts returning from space have an increased coarsening of the skin (characterized by a decreased hydration of the stratum corneum) that is consistent with astronauts' complaints of skin pruritis and dryness (14). In these studies, skin dryness and pruritis were found in animals exposed to electron radiation.

Pigs serve as a model for the study of radiation effects on the lungs because their lungs are anatomically and physiologically similar to human lungs. In addition, due to their size, they are a practical laboratory animal model (15). In this study, doses from 7.5 Gy to 25 Gy resulted in significant toxicity to internal organ systems, with pulmonary toxicities being most pronounced. One of three animals irradiated with 7.5 Gy, one of three animals irradiated with 12.5 Gy, and all three animals irradiated with 25 Gy experienced a radiation-associated pneumonopathy with a radiographic pattern that followed the pattern of dose deposition. Animals that received 7.5 to 25 Gy also experienced immunological dysfunction as seen in the increased DTH response to PHA and subsequent mycoplasmal infection in the animals treated with 25 Gy. Although the decreased DTH response and the mycoplasmal infection may have been due to an immunological dysfunction created by the exposure to radiation, in the animals that were found to have pneumonia, the decreased DTH response may also have been attributable to the secondary mycoplasmal infection. Further investigation is necessary to determine the exact cause for the immunological dysfunction. Since the majority of animals that developed pneumonia were in the highest dose group, it is speculated that it was actually the exposure to radiation itself that caused the immunological dysfunction. The stress associated with the irradiation procedure is not believed to be the cause because all animals underwent the exact same procedure, but clinical signs developed quickly and most aggressively in the animals that had received the highest dose (25 Gy) of radiation.

M. hyopneumoniae is a widespread and endemic microbial agent found in most swine herds. It can be present within a swine population at undetectable levels for an extended period and generally results in a subclinical or uncomplicated infection except when other infectious agents or stressors are present that increase the susceptibility to disease. It is rarely seen clinically in the laboratory animal environment. *M. hyopneumoniae* colonizes the airway of any age pig, generally causing a mild disease displayed by coughing, dyspnea and fever (16). In the case of the animals in this study, it is speculated that radiation caused an immunological dysfunction and pulmonary pathology, resulting in increased susceptibility to infection with *M. hyopneumoniae*. The pulmonary pathology observed radiographically

and histologically was consistent with the lesions observed in humans diagnosed with radiation pneumonitis (i.e., ground glass opacities on CT scan, edema, inflammatory cell accumulation, pleural thickening and fibrosis on histology), with some features also consistent with *M. hyopneumoniae* infection (i.e., cranioventral lung consolidation on necropsy, bronchointerstitial pneumonia on histology) (15, 17, 18). Functional lung damage in pigs likely occurs after a threshold volume of lung is exposed to radiation (19). It appears that a cooperative effect between the highly inhomogeneous dose distribution and immunological dysfunction may have led to pulmonary toxicities that occur in a shorter time after irradiation and at a much lower mean lung dose than has been reported previously (15, 19). These experiments, along with the results for humans irradiated in clinical trials using inhomogeneous irradiation fields (20, 21), suggest that pneumonitis could be the result of a combination of high doses to a small portion of the lung along with low radiation doses to a large volume of the lung.

The neurological signs seen in one of three pigs exposed to 25 Gy may have been related to central nervous system toxicity, which has been described in humans after radiation therapy (i.e. lethargy, ataxia, lower-extremity weakness and hyperreflexia) (17). In humans, radiation myelopathy is typically a late complication, occurring 9 to 15 months after radiation therapy (17). This effect may also be related to the highly inhomogeneous dose distribution delivered to the animals.

The effects observed in WBC, lymphocyte and neutrophil counts are likely due to both primary and secondary radiation effects. At 24 h postirradiation the effects on blood cell counts are likely to be due to the cell killing effects of radiation. However, some of the other effects observed in WBC at later times may be due to both primary and secondary radiation effects. For example, the elevation of WBC counts at 30 days after exposure to 25 Gy may be evidence of mycobacterial infection occurring in the third and fourth weeks postirradiation, which is suggestive, although not definitively diagnostic, of a clinical picture in which radiation pneumonitis is followed by a superinfection with mycoplasmal pneumonia. Thus the significant elevation in WBC count at day 30 in the group of pigs irradiated with 25 Gy was associated with superinfection that occurred at that time and also represents a secondary, radiation-associated WBC alteration. In conclusion, using total doses, doses rates and patterns of dose distribution that are compatible with potential astronaut exposure to SPE radiation, animals experienced significant toxicities to both superficial and deep organs. Further research is necessary to determine whether SPE-like proton radiation will cause similar or even greater toxicity to the skin and internal organs. Finally, these effects model a relatively superficial SPE. It is predicted, however, that more severe effects may occur after exposure to a “harder” SPE because of the increased amount of radiation dose received in deeper tissues compared to the current model of 6 MeV electrons, which is equivalent to the dose–depth characteristics of 50 MeV protons.

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FIG. 1. Simulated dosimetry. Panel A: Organs were identified on cross-sectional imaging (CT scans). Panel B: Doses were simulated for 6 MeV electron irradiation of a pig using a Monte Carlo-based simulation algorithm (Varian Medical Systems, Palo Alto, CA). Organ dose–volume histograms (panel C) and dose statistics (panel D) were determined using the organ volumes and simulated dose distribution for the 12.5-Gy dose cohort.

FIG. 2.

Radiation-Induced skin hyperpigmentation and internal organ complications. Panel A: H&E-stained sections showing normal, unirradiated skin from a Yucatan minipig (left) and skin from a Yucatan minipig 7 days after irradiation with 12.5 Gy (right). The biopsy from the irradiated minipig (right) demonstrates enlargement of melanocytes and increased basilar keratinocyte pigmentation. Panel B: Quantification of pigmentation as a function of dose displayed as minimum, maximum and mean. Animals displayed hyperpigmentation changes that were most pronounced by 14 days postirradiation. The degree of hyperpigmentation changes increased with increasing dose. Panel C: Additional skin toxicities are presented as the percentage of animals displaying each effect ($n = 3$), except for wound healing, which is represented as the percentage of grade 5 (necrotic/nonhealing) biopsy sites compared to the total number of biopsy sites ($n = 48$). Grade 1 dry skin was considered mild, with fine thin scales, slightly rough to touch with no fissures or inflammation present. Grade 2 dry skin was considered moderate, consisting of scales with definite margins, rough to touch with few fissures present but shallow and not hemorrhagic. Grade 1 alopecia was characterized by thinning or patchy hair loss. Ulcerations were considered Grade 1 when they were superficial and less than 2 cm in size.

FIG. 3. Radiation-induced skin complications. Panel A: H&E-stained section demonstrating multiple necrotic keratinocytes (white arrows) with the characteristic dyskeratotic cytoplasm and pyknotic nuclei in the epidermal layer of a pig irradiated with 10 Gy, 14 days after irradiation. Scale bar, 20 μ M. Panel B: Necrotic keratinocytes were scored on at least 10 high-power fields for three animals per dose; the average numbers of necrotic keratinocytes ($\pm$ SEM) per mm of skin biopsy are presented.

FIG. 4. Radiation-induced pulmonary changes after superficial irradiation with 6 MeV electrons. Panel A: Representative image from a non-contrast CT scan performed 2 months after receiving 12.5 Gy radiation in an animal that experienced respiratory symptoms, including chronic dry coughing. The arrow shows an area of ground glass opacity, and the arrowhead shows atelectasis and bronchiectasis in the middle lobe. A clinical diagnosis of radiation-associated pneumonopathy was made on the basis of these findings. Panel B: H&E-stained section showing acute lung injury in an animal that had received 25 Gy from 6 MeV electrons. Combined infectious and radiation-associated pneumonitis can be observed as a peribronchial increase in bronchus-associated lymphoid tissue (thin black arrow), septal edema, mononuclear infiltrate (black arrowhead) and broncho-interstitial pneumonia with eosinophilic alveolar infiltrate (thick black arrow).

FIG. 5.

Radiation induced white blood cell count alterations. Automated complete blood counts were performed on all animals prior to radiation exposure (Pre) and on the indicated day after irradiation. Only the dose groups showing statistically significant changes (compared to the preirradiation count) in total white blood cells (panel A), lymphocytes (15-Gy dose group, panel B, and 25-Gy dose group, panel C), and neutrophils (panel D) are shown. Changes in cell counts were compared to the preirradiation counts and were considered significant at $P < 0.05$ using one-way ANOVA (*).

FIG. 6.

Delayed-type hypersensitivity (DTH) responses are increased in animals exposed to SPE-like radiation. Local reaction to serial phytohemagglutinin injections were measured in all animals. The animals exposed to 7.5, 12.5, 15 and 25 Gy of electron radiation showed statistically significant increases in DTH response compared to the sham-irradiated animals (*) at 7 days. The data represent the average measured response (in mm) $\pm$ SEM and were considered significant at $P < 0.05$, using the Student's t test.

TABLE 1

Description of the SPE Simulation and Procedural Timeline

Dose	Dose rate	Sample size	CBC analysis	PHA skin reactivity testing	DTH response measured	Skin biopsy/histopathology	Necropsy
Sham	-	2	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	30 days
Sham	-	1	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	8 months
5 Gy	1.7 Gy/h	3	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	30 days
7.5 Gy	2.5 Gy/h	3	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	30 days
10 Gy	3.3 Gy/h	3	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	30 days
12.5 Gy	4.2 Gy/h	2	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	30 days
12.5 Gy	4.2 Gy/h	1	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	9 months
15 Gy	5.0 Gy/h	3	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	30 days
25 Gy	8.3 Gy/h	3	4 h, 24 h, 7 days, 14 days, 30 days	-2 days, 5 days, 12 days, 28 days	0 days, 7 days, 14 days, 30 days	0 days, 7 days, 14 days, 30 days	30 days