Paper

A DOSE-DEPENDENT HEMATOLOGICAL EVALUATION OF WHOLE-BODY GAMMA-IRRADIATION IN THE GÖTTINGEN MINIPIG

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Abstract—**There is a great deal of interest in the establishment of a standardized animal model for the acute radiation syndrome to allow development of diagnostic approaches and countermeasure treatments following radiological terrorist events. Due to physiological, anatomical, and biochemical similarities to humans, the minipig is an attractive large animal model for evaluating countermeasure efficacy. This study was conducted in order to aid in the establishment of the minipig, and the Göttingen minipig in particular, as an animal model for the hematopoietic acute radiation syndrome. Animals were exposed whole-body to ^{60}Co at doses of 0 (sham control), 0.25, 0.5, 0.75, 1.0, and 2.0 Gy, and hematological parameters followed in time from pre-irradiation to post-irradiation Day 7. Following irradiation, a dose-dependent decrease in total white blood cells was observed, which was determined to be statistically different as compared to control animals at all dose levels above 0.25 Gy at 24 h post-irradiation. Similarly, a dose-dependent reduction in both absolute lymphocyte count and absolute neutrophil count occurred by the earliest time point measured for all exposed animals. A significant decrease in platelets was observed at post-irradiation Day 7 in animals exposed only at the highest (2.0 Gy) level. The platelet-to-lymphocyte ratio generated for exposures ranging from 0.25–2.0 Gy was able to differentiate response between high and low exposure levels even at 7 d post exposure. In conclusion, the present study supports the development of the Göttingen minipig as a suitable large animal model to study radiation-induced hematopoietic syndrome.**
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Key words: biological indicators; gamma radiation; laboratory animals; whole body irradiation

INTRODUCTION

Realistic concerns over nuclear accidents and threats of terrorism have spurred efforts to improve methods to protect military personnel, the general population, first responders, and other service groups from the health hazards associated with exposure to ionizing radiation. The possibility of a large-scale incident in which thousands of people are exposed will require improved methods to assess exposures quickly. A number of different techniques have been reported for estimation of radiation exposure, including detection of induced chromosomal abnormalities (Gotoh et al. 2005) and electron paramagnetic resonance in teeth (Kleinerman et al. 2006). Classically, a quick and approximate classification of acute radiation syndrome severity in man has been based primarily on time to emesis—the higher the dose, the sooner the victim vomits (Anno et al. 1989). Much effort has focused on establishment of protocols for medical management of radiation injuries based on hematopoietic changes for biodosimetry (Fliedner et al. 2007; Cronkite 1967; Cronkite and Fliedner 1972; Goans et al. 1997). The characterization work described in the present study was designed to evaluate the early hematopoietic response to whole-body irradiation in the Göttingen minipig, a large animal model currently being explored as an appropriate model to represent man. The wide range of whole-body irradiation doses evaluated here should greatly enhance the approximation between the hematopoietic response in the Göttingen minipig and the human equivalent.

The pig (*Sus scrofa domestica*) has played a critical role in the scientific community as a non-rodent alternative to the dog or non-human primate. The pig shares many of the same basic anatomy, physiology, biochemistry, pathology, and pharmacology characteristics as a human (Nunoya et al. 2007; Donnadieu-Claraz et al. 1999). The miniature pig (minipig), in particular, has gained popularity in research studies due to the ease of handling the smaller size compared to the domestic pig and well characterized and controlled genotypes. For example, the Göttingen minipig is well socialized, is barrier bred, is

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maintained under a defined and controlled environment, and has a defined microbiological health status.

Moroni et al. (2011a and b) has reported hematological changes in the Göttingen minipig for whole body irradiation doses spanning the lethal range of exposure LD_{10} to LD_{100} at 30 d (approximately 1.6–2.0 Gy). These studies support the idea that the pathophysiology of acute radiation sickness in the Göttingen minipig is similar to that observed in humans, non-human primates, and canines, particularly in terms of hematological dynamics. To appreciate fully the utility of the minipig as an animal model for radiation countermeasure development, it is important to be able to distinguish dose-related changes in key hematological symptoms, including neutropenia, thrombocytopenia, and anemia. Hematopoietic cells are highly sensitive to radiation damage even at relatively low levels of exposure, and understanding the temporal appearance of each of the symptoms with progressively increasing doses of radiation is invaluable in understanding the animal model. Although prior studies have provided a solid beginning to characterization of the minipig, the pattern of hematological changes across a wide range of irradiation exposure has not been described. The data described in this study were designed to fill that need.

MATERIALS AND METHODS

Animals

Male Göttingen minipigs, approximately 3–4 mo of age, were obtained from Marshall BioResources (North Rose, NY); animals weighed approximately 5–7 kg at shipment. Animals were housed individually in modular floor pens measuring 1.1 m^2. Pens were attached in units, allowing pigs to see and touch neighboring animals. Autoclaved fir wood shavings were provided for bedding and were changed at least daily. Individual water lines with lixit valves were secured to pen walls; polyethylene balls (Bio-Serv, Frenchtown, NJ) were provided for enrichment. Animals were fed Lab Diet K599 Certified Lab Minipig Grower and Maintenance feed (PMI Nutrition International, LLC, Brentwood, MO) in a ration based on age twice daily. Certified fruit crunchies (Bio-Serv, Frenchtown, NJ) and locally procured apples and miniature marshmallows were used for positive reinforcement. The light cycle was 12 h light and 12 h dark, and temperature and humidity were as recommended for minipigs provided bedding: 18–22°C and 35–65%, respectively. Animals were allowed to acclimate fully to the facility for at least 2 wk prior to irradiation. Temperature and identification transponders were implanted subcutaneously (BMDS IPTT-300, Seaford, DE) during the acclimation period. Animal protocols were approved by the Institutional Animal Care and Use Committee at Battelle, Pacific Northwest Division. The facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, is registered with the USDA, and holds an Office of Laboratory Animal Welfare assurance.

Irradiation

Radiation exposure and dosimetry conditions followed recommendations set forth in Report 30 of the International Commission on Radiation Units and Measurements (ICRU 1979). In brief, anesthetized animals were irradiated using a nominal 173,900 GBq ^{60}Co source contained within an automated irradiator. The source selected was moved into place using a pneumatic system to a position 170-cm above the floor within a collimator that provided an approximate 30-degree solid angle beam. Prior to irradiating live animals, the correlation between internal center-line dose measurements using LiF:Mg:Ti "chipstrate" dosimeters in a euthanized animal and external exposure using a Capintec ionization chamber were established. Two separate dosimetric methods were used so results could be compared and uncertainties in the accuracy of the measured doses known to a high degree. Chipstrate dosimeters were calibrated in a National Institute of Standards and Technology (NIST)-traceable ^{60}Co beam using tissue-equivalent plastic for buildup to accommodate a reference to absorbed dose to tissue.

Exposure conditions were established to provide a known absorbed dose rate at the geometric center of a euthanized animal representative of the study population. Calibrated dosimeters were located at 14 locations at different depths along the length of the animal. The data indicated dose uniformity across the animal within ± 10% relative to body center, with the exception of the deepest tissue location at the hips, which had a maximum of approximately 13% nonuniformity. Because the minipig study population varied in body weight, body length, and width measured at multiple locations (hips, above the hips, body center, shoulders), conditions of irradiations performed at a constant distance from the source would result in inconsistent absorbed dose along the midline of the body. Thus, the distance of each minipig from the source was adjusted to account for the difference in beam attenuation relative to the euthanized reference minipig. Specifically, the average width of each minipig determined at multiple locations and lengths were measured immediately prior to irradiation and area calculated. Using this minipig area correlation with average depth, as opposed to using correlation with pig weight, was determined to result in better dose reproducibility.

For irradiation, anesthetized animals were restrained in a hammock-style sling with legs positioned under the animal and placed on a rotating platform. The sling and

restrained animal were rotated by a remotely operated platform turntable. To achieve the desired target dose rate of 400 mGy min^{-1}, animals in slings were positioned approximately 90 cm from the source. Due to this close distance and the need to use continuous rotation during exposures, the outer edges of the gamma-ray beam were shaped using aluminum attenuators. This provided dose uniformity across the entire body. Whole body gamma-irradiation doses ranged from 0.25–2.0 Gy at a dose rate of 400 mGy min^{-1}. Day of irradiation was considered day 0.

Experimental procedures

Group sizes per exposure were as follows: sham (control) animals, n = 12; 0.25 Gy, n = 4; 0.5 Gy, n = 4; 0.75 Gy, n = 4; 1.0 Gy, n = 8; and 2.0 Gy, n = 4. Animals were anesthetized with ketamine (33 mg kg^{-1}) and acepromazine (1.1 mg kg^{-1}) by intramuscular (IM) injection and transported to the irradiator in covered animal crates. Following irradiation, animals were observed twice daily and clinical signs recorded. Body weight and temperature (from the implanted transponder) were measured daily. Analgesia (carprofen 2–3 mg kg^{-1} orally, twice a day) was provided to each animal within 24 h following irradiation and continued for the duration of the study. Although clearly defined endpoint criteria were established for early moribund euthanasia, no animal required euthanasia prior to the scheduled end of study. At the completion of the study period, animals were anesthetized with ketamine (33 mg kg^{-1}) and acepromazine (1.1 mg kg^{-1}) by IM and euthanized with sodium pentobarbital (150 mg kg^{-1}) by intravenous injection.

Blood was collected from the cephalic or cranial epigastric veins for assessment of hematological and clinical chemistry parameters from animals that were sedated using midazolam (0.1–0.5 mg kg^{-1}) by subcutaneous (SC) or IM injection. A single animal became resistant to midazolam and was sedated using acepromazine (1.1 mg kg^{-1}) by SC or IM injection. Complete hematological assays were performed using an Advia 120 (Siemens Medical Solutions Diagnostics, Tarrytown, NY) hematology analyzer on whole blood collected in potassium-ethylenediaminetetraacetic acid. All blood analyses were completed within 3 h of collection. Baseline collections from age-matched (non-irradiated) animals were used to establish a laboratory historical database.

Statistical analyses

For each animal, hematological parameters were obtained pre- and post-irradiation. An average value was calculated for each exposure group for each sampling time point, and averages and standard deviations are reported. Statistically significant differences between group means at individual time points were determined by one-way ANOVA ($p < 0.05$) and Tukey's HSD Post Hoc test as appropriate.

RESULTS

Passive dosimeter and ionization chamber measurements conducted using a euthanized minipig showed an absorbed dose rate of 0.39 Gy min^{-1} at the center of the animal. The uncertainty associated with the delivered free-field dose rate is 2.3% at the 95% confidence level, and the uncertainty associated with the Gy per coulomb of the transfer standard ionization chamber placed at animal center is estimated at 3% at the 95% confidence level. The uncertainty accompanying the use of minipig area to correlate dose depth was estimated to be ± 5% with a rectangular probability distribution. The uncertainty in the use of the tissue depth curve was estimated to be ± 3% with a rectangular probability distribution. The uncertainty in the dose influence due to uncertainty in source per animal distance was estimated to be ± 0.7% with a rectangular probability distribution. Therefore, the maximum uncertainty in the stated absorbed dose value at the center location is calculated to be 7.5% at the 95% confidence level and is in terms of the traceability to national standards (i.e., the accuracy of the measured doses). The variation of this central dose among animals within the study group at the same dose level was calculated to be approximately ± 7.0% at the 95% confidence level.

Hematological parameters were determined for all animals pre- and post-irradiation. For reference, this laboratory's historical values are provided as a range of the average ± standard deviation of n = 140 individual points from age-matched, non-irradiated animals (shaded areas, all figures). This laboratory's baseline values correlate well with those reported by others working with the same minipig model (Ellegaard et al. 1995; Moroni et al. 2011c). A pilot study was conducted to evaluate any variations in measured hematological values when collected serially from the epigastric versus cephalic veins. Although matched collections showed expected fluctuations, no consistent differences or trends were observed between intra-animal matched samples (data not shown).

Overall, a dose-dependent decrease in total white blood cells (WBC) was observed for all irradiated groups of animals beginning with the first blood sample collected at 24 h post irradiation (see Fig. 1 and Table 1). In comparison to sham-irradiated (control) animals, statistically significant reductions in WBC counts were detected at 24 h post-irradiation for animals at all dose levels except the 0.25 Gy group. Similarly, at 24 h post exposure, statistically significant reductions in WBC counts were evident for all irradiated animals at 24 h in comparison to animals

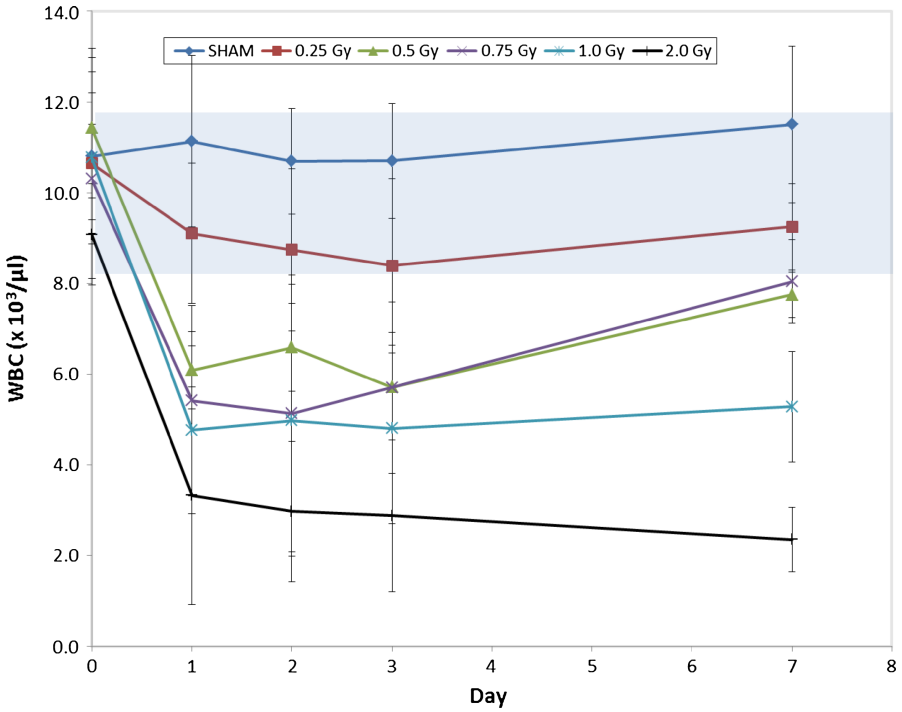

Fig. 1. Average and standard deviation of WBC count ($\times 10^3$ μL^{-1}) over time from pre-irradiation (day 0) to post-irradiation day 7 for whole-body exposures to 0 (sham control), 0.25, 0.5, 0.75, 1.0, and 2.0 Gy gamma irradiation. The shaded bar represents the range of the average and standard deviation of n = 140 individual data measurements from age-matched non-irradiated animals.

at the 0.25 Gy exposure level and between animals irradiated at 0.5 Gy versus 2.0 Gy (Table 1). At the lowest irradiation dose (0.25 Gy), WBC counts were not statistically different from control animals by 48 h post exposure. In contrast, WBC counts for the 1.0 and 2.0 Gy exposure groups remained significantly reduced compared to sham irradiated control animals (Table 1) for the duration of the study period (7 d). The WBC counts for animals exposed at 0.5 and 0.75 Gy had nearly returned to the historic range for age-matched (non-exposed) animals by post exposure day 7.

A dose dependent reduction in absolute lymphocyte counts occurred by 24 h post irradiation (Fig. 2), the earliest point measured. In comparison to control animals, absolute lymphocyte counts for all irradiated groups of animals continued to be significantly lower at 7 d post exposure, and for exposures greater than 0.25 Gy, values were outside the historic range for age-matched (non-exposed) animals (Table 2). Although lymphocyte counts for 0.25 Gy animals at 7 d post exposure were statistically different compared to the concurrent control animals for this study, values were within the historic range. An approximate 60% decline in blood lymphocyte counts was evident at 24 h post exposure in the 0.5 Gy group, with even greater decreases occurring at higher dose levels. For the group of animals exposed at the lethal dose level (2.0 Gy), absolute lymphocyte counts were less than 1,000 cells μL^{-1} at 24 h post exposure, which is more than an 80% decrease from baseline values (roughly 5,900 cells μL^{-1}) measured in the same animals.

Table 1. White blood cell count data.

Exposure dose (Gy)	Day 0 ($\times 10^3$ per µL)	24 h ($\times 10^3$ per µL)	7 Day ($\times 10^3$ per µL)
0	10.8 ± 1.3	11.1 ± 1.7	11.5 ± 1.7
0.25	10.6 ± 1.9	9.1 ± 1.0	9.2 ± 1.6
0.5	11.4 ± 0.9	6.1 ± 0.5[ab]	7.8 ± 1.5
0.75	10.3 ± 1.9	5.4 ± 0.9[ab]	8.0 ± 3.6
1.0	10.8 ± 2.1	4.8 ± 1.2[ab]	5.3 ± 0.7[a]
2.0	9.1 ± 1.7	3.3 ± 0.7[abc]	2.4 ± 0.5[abcd]

[a]Statistically different from sham control animals measured at the same time period.
[b]Statistically different from 0.25 Gy animals measured at the same time period.
[c]Statistically different from 0.50 Gy animals measured at the same time period.
[d]Statistically different from 0.75 Gy animals measured at the same time period.

Similarly, a dose-dependent decrease in absolute neutrophil counts was detected in all irradiated animals immediately post irradiation, although to a lesser degree than that observed with the absolute lymphocyte count levels. For example, at 7 d post exposure, absolute neutrophils were roughly 40% of the baseline (day 0) value for the highest exposure group (1,400 cells μL^{-1} compared to an initial 3,100 cells μL^{-1}). As a percent of the total white blood cell population, a statistically significant 20–30% increase in neutrophils was evident at 1.0 Gy and higher by 24 h post exposure (Fig. 3, Table 3). Although neutropenia (<500 cells μL^{-1}) was not observed at any exposure in the current study, prior work by Moroni et al. (2011a and b)

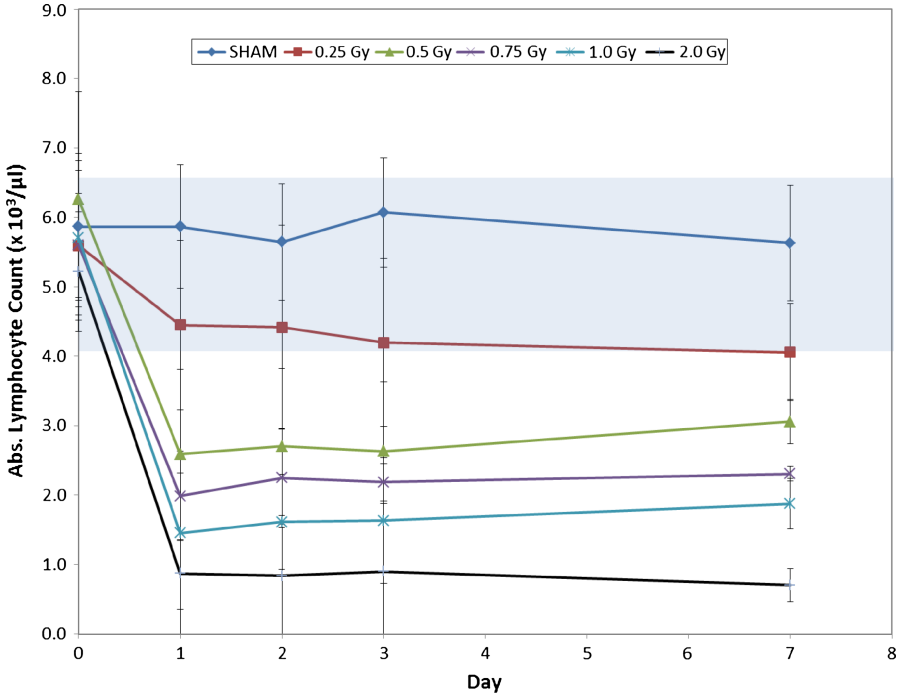

Fig. 2. Average and standard deviation of absolute lymphocyte count ($\times 10^3$ μL) over time from pre-irradiation (day 0) to post-irradiation day 7 for whole-body exposures to 0 (sham control), 0.25, 0.5, 0.75, 1.0, and 2.0 Gy gamma irradiation. The shaded bar represents the range of the average and standard deviation of $n = 140$ individual data measurements from age-matched non-irradiated animals.

indicates neutropenia is observed 14–17 d post exposure, which is outside the monitoring period used here.

Compared to baseline (day 0) values, changes in circulating red blood cells (RBCs) were not evident within the initial 7 d post irradiation (Table 4), although an initial slight but non-significant decrease in reticulocyte levels was noted (data not shown). For platelets, a significant decrease was observed at 7 d post-exposure in animals exposed at the highest level (2.0 Gy) in comparison to the control and all other irradiated animals (Fig. 4). No other measured hematological component was remarkable.

It has been postulated that hematological ratios (neutrophil-to-lymphocyte, neutrophil-to-platelet, and platelet-to-lymphocyte) may have utility as diagnostic indicators for accessing acute radiation exposure. Of these, dose-dependent increases in the neutrophil-to-platelet and platelet-to-lymphocyte ratios were evident for all irradiated groups of animals. For example, as illustrated for the platelet-to-lymphocyte ratio (Fig. 5), an increase in the ratio was observed by 24 h post-exposure for all irradiated animals compared to control animals, with the increase attaining statistical significance at exposures of 0.75 and above (Table 5). As late as 7 d post-exposure, the platelet-to-lymphocyte ratio remained statistically elevated for animals exposed at the lethal level (2.0 Gy) compared to the control, 0.25 Gy, and 0.5 Gy groups.

Table 2. Absolute lymphocyte count data.

Exposure dose (Gy)	Day 0 ($\times 10^3$ per μL)	24 h ($\times 10^3$ per μL)	7 Day ($\times 10^3$ per μL)
0	5.9 ± 0.8	5.9 ± 0.8	5.6 ± 0.6
0.25	5.6 ± 1.2	4.4 ± 0.7[a]	4.1 ± 0.8[a]
0.5	6.3 ± 1.0	2.6 ± 0.3[ab]	3.1 ± 0.3[a]
0.75	5.6 ± 0.3	2.0 ± 0.1[ab]	2.3 ± 0.4[ab]
1.0	5.7 ± 0.9	1.5 ± 0.4[abc]	1.9 ± 0.4[abc]
2.0	5.2 ± 1.0	0.9 ± 0.2[abcd]	0.7 ± 0.1[abcde]

[a]Statistically different from sham control animals measured at the same time period.
[b]Statistically different from 0.25 Gy animals measured at the same time period.
[c]Statistically different from 0.50 Gy animals measured at the same time period.
[d]Statistically different from 0.75 Gy animals measured at the same time period.
[e]Statistically different from 1.0 Gy animals measured at the same time period.

DISCUSSION AND CONCLUSION

There is much interest in the development of a standardized animal model for the hematopoietic or bone marrow acute radiation syndrome to allow comparison of efficacy in prophylaxis, mitigation, and treatment of radiation injury consistent with the FDA "Animal Rule" framework. The majority of radiation studies on the hematopoietic syndrome have used the inbred mouse, canine, and non-human primate (Williams et al. 2010). The Göttingen minipig represents an attractive alternative large animal model to the canine and non-human primate for several reasons, including similarities in anatomy and physiology and ease of handling. For consideration under the "Animal Rule," one essential requirement is that there

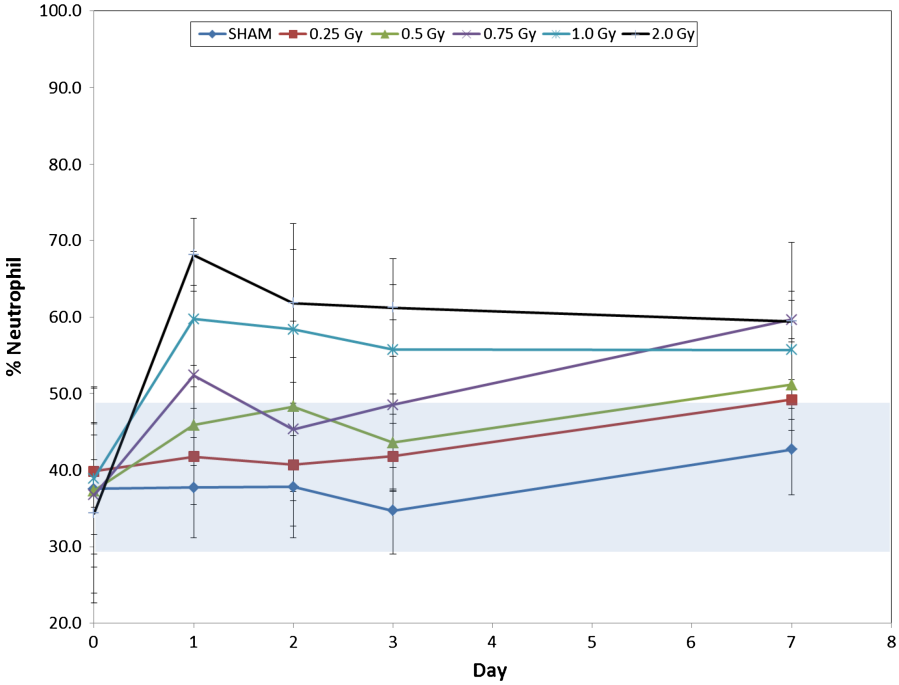

Fig. 3. Average and standard deviation of neutrophils expressed as percent of the white blood cell count (%) over time from pre-irradiation (day 0) to post-irradiation day 7 for whole-body exposures to 0 (sham control), 0.25, 0.5, 0.75, 1.0, and 2.0 Gy gamma irradiation. The shaded bar represents the range of the average and standard deviation of $n = 140$ individual data measurements from age-matched non-irradiated animals.

must be a reasonably well understood pathophysiological mechanism demonstrated in a sufficiently well characterized animal model for predicting the response in humans. The work described here was undertaken in order to aid in the establishment of the minipig, and the Göttingen minipig in particular, as an animal model for acute radiation hematopoietic syndrome.

Work by Moroni et al. (2011a and b) suggests the $LD_{50/30}$ value in the Göttingen minipig is between 1.7–1.9 Gy and that the acute radiation syndrome pathophysiology closely parallels what has been observed in humans and large animal models. The preceding work with minipigs has correlated a number of hematopoietic parameters with mortality, including platelet cut-off values and cumulative number of days of thrombocytopenia. Blakeley et al. (2010) have demonstrated four biomarkers (lymphocytes, neutrophils, ratio of neutrophils-to-lymphocytes, and serum amylase activity) as discriminant for pre- versus post-irradiation levels in the non-human primate. Along these lines, Moroni et al. (2011b) demonstrated that the neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios are particularly important as prognostic indicators of irradiation in the minipig. Given this, the range of doses employed in the present study can be used to expand greatly on these predictive signs of radiation exposure. For example, the platelet-to-lymphocyte ratio generated for exposures ranging from 0.25–2.0 Gy (Fig. 5) can differentiate response between high and low exposure levels. Importantly, the platelet-to-lymphocyte ratio illustrated in Fig. 5 appears to discriminate between dose groups even at 7 d post exposure, particularly for low versus high (lethal) exposures.

Table 3. Neutrophils as a percentage of total blood cell count.

Exposure dose (Gy)	Day 0 (%)	24 hr (%)	7 Day (%)
0	37.6 ± 5.6	37.7 ± 6.0	42.7 ± 9.3
0.25	39.9 ± 4.3	41.8 ± 2.6	49.2 ± 3.2
0.5	37.3 ± 6.4	45.9 ± 6.0	51.2 ± 6.8
0.75	36.8 ± 11.4	52.4 ± 10.1	59.7 ± 15.6
1.0	38.9 ± 8.5	59.8 ± 7.6[abc]	55.7 ± 6.2
2.0	34.4 ± 6.4	68.1 ± 2.7[abcd]	59.5 ± 6.1

[a]Statistically different from sham control animals measured at the same time period.
[b]Statistically different from 0.25 Gy animals measured at the same time period.
[c]Statistically different from 0.50 Gy animals measured at the same time period.
[d]Statistically different from 0.75 Gy animals measured at the same time period.

Table 4. Red blood cell count.

Exposure dose (Gy)	Day 0 ($\times 10^6$ per μL)	24 h ($\times 10^6$ per μL)	7 Day ($\times 10^6$ per μL)
0	9.0 ± 0.5	8.3 ± 0.4	8.9 ± 0.6
0.25	9.0 ± 0.9	8.4 ± 1.0	8.6 ± 0.9
0.5	9.8 ± 0.5	8.9 ± 0.5	9.1 ± 0.6
0.75	8.0 ± 0.5	7.7 ± 1.0	8.3 ± 0.3
1.0	8.7 ± 0.5	7.9 ± 0.6	8.4 ± 0.6
2.0	8.7 ± 0.5	8.6 ± 0.5	8.5 ± 0.7

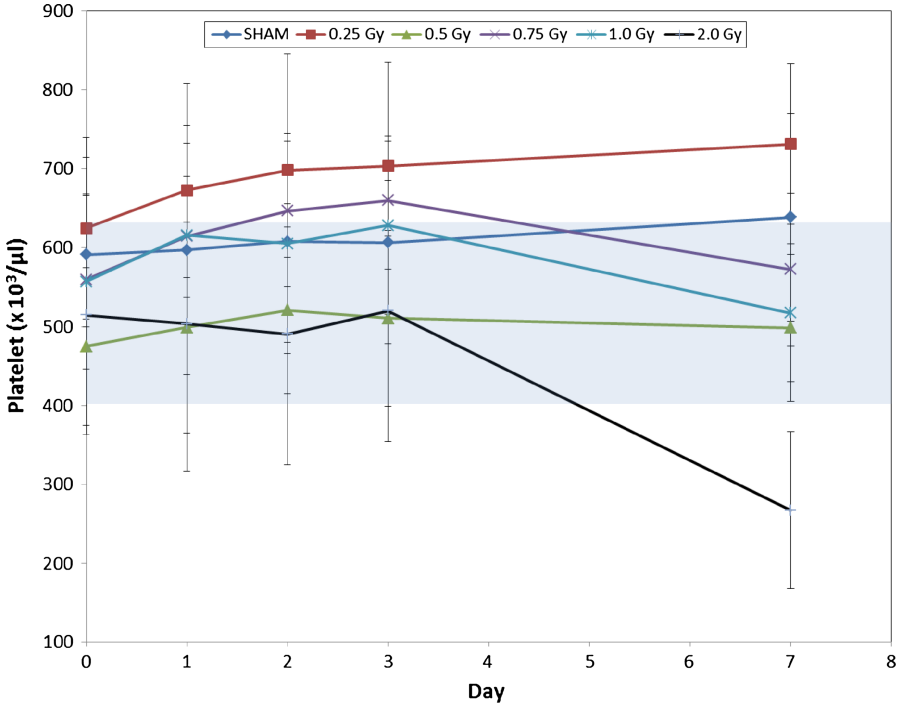

Fig. 4. Average and standard deviation of platelet count ($\times 10^3$ μL^{-1}) over time from pre-irradiation (day 0) to post-irradiation day 7 for whole-body exposures to 0 (sham control), 0.25, 0.5, 0.75, 1.0, and 2.0 Gy gamma irradiation. The shaded bar represents the range of the average and standard deviation of $n = 140$ individual data measurements from age-matched non-irradiated animals.

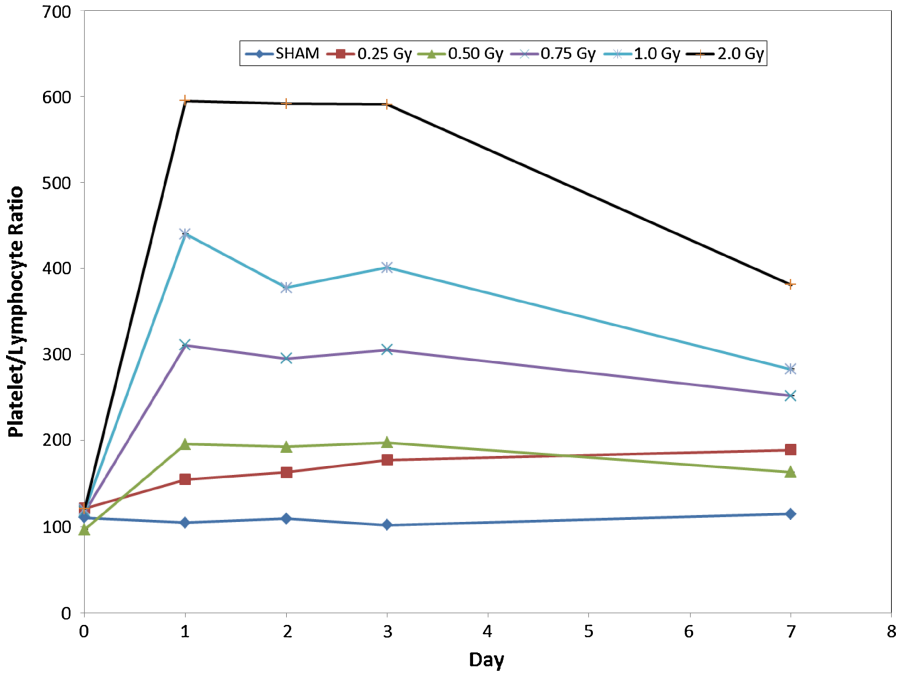

Fig. 5. Average platelet-to-lymphocyte ratio over time from pre-irradiation (day 0) to post-irradiation day 7 calculated for animals exposed to 0 (sham control), 0.25, 0.5, 0.75, 1.0, and 2.0 Gy whole-body gamma irradiation. Ratios were calculated for each individual animal and averaged per group.

Table 5. Platelet-to-lymphocyte ratio as function of whole-body dose.

Exposure Dose (Gy)	Day 0	24 h	48 h	7 Day
0	110 ± 31	104 ± 32	109 ± 27	115 ± 23
0.25	121 ± 53	155 ± 39	163 ± 41	189 ± 66
0.5	97 ± 32	196 ± 47	193 ± 15	163 ± 38
0.75	118 ± 23	311 ± 58[a]	295 ± 82	252 ± 36
1.0	120 ± 27	440 ± 106[abc]	378 ± 72[ab]	283 ± 54[a]
2.0	121 ± 9	595 ± 41[abcd]	592 ± 151[abcde]	381 ± 122[abc]

[a]Statistically different from sham control animals measured at the same time period.
[b]Statistically different from 0.25 Gy animals measured at the same time period.
[c]Statistically different from 0.50 Gy animals measured at the same time period.
[d]Statistically different from 0.75 Gy animals measured at the same time period.
[e]Statistically different from 1.0 Gy animals measured at the same time period.

Together, the studies reported here and work by others indicate the promising utility of the minipig as an alternative large animal model for radiation countermeasure development, consistent with the FDA "Animal Rule." Clearly further work is necessary and warranted to characterize fully the natural history of response in the minipig with and without concurrent supportive care measures.

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