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

Karla D. Thrall,* Jamie Lovaglio,* Mark K. Murphy,† Renee N. Cataneo,‡ Anirudh Chaturvedi,‡ Mayur Mundada,‡ Urvish Patel,‡ and Michael Phillips‡

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 60Co 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 to 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.

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...
proved by the Institutional Animal Care and Use Committee
during the acclimation period. Animal protocols were
implanted subcutaneously (BMDS IPTT-300, Seaford, DE)
irradiation. Temperature and identification transponders were
used for positive reinforcement. The light cycle was
and locally procured apples and miniature marshmallows
provided for enrichment. Lixit valves were secured to pen walls; polyethylene balls
and were changed at least daily. Individual water lines with
Autoclaved fir wood shavings were provided for bedding.

MATERIALS AND METHODS

Animals
Male Göttingen minipigs, approximately 3–4 mo of
time, 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 m². 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 Interna-
tional, 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 ap-
proved by the Institutional Animal Care and Use Committee
at Battelle, Pacific Northwest Division. The facility is
accredited by the Association for Assessment and Accredi-
tation of Laboratory Animal Care International, is regis-
tered with the USDA, and holds an Office of Laboratory
Animal Welfare assurance.

Irradiation
Radiation exposure and dosimetry conditions fol-
lowed recommendations set forth in Report 30 of the
International Commission on Radiation Units and Mea-
surements (ICRU 1979). In brief, anesthetized animals
were irradiated using a nominal 173,900 GBq ⁶⁰Co 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 ex-
ternal exposure using a Capintec ionization chamber were
established. Two separate dosimetric methods were used so
results could be compared and uncertainties in the accu-

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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 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 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

**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.

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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 \( \times 10^3 \) per \( \mu L \), which is more than an 80% decrease from baseline values (roughly 5,900 cells \( \times 10^3 \) per \( \mu L \)) measured in the same animals.

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 \( \times 10^3 \) per \( \mu L \)) compared to an initial 3,100 cells \( \times 10^3 \) per \( \mu L \). 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 \( \times 10^3 \) per \( \mu L \)) was not observed at any exposure in the current study, prior work by Moroni et al. (2011a and b)

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Table 1. White blood cell count data.

<table>
<thead>
<tr>
<th>Exposure dose (Gy)</th>
<th>Day 0 ( (\times 10^3 ) per ( \mu L )</th>
<th>24 h ( (\times 10^3 ) per ( \mu L )</th>
<th>7 Day ( (\times 10^3 ) per ( \mu L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.8 ± 1.3</td>
<td>11.1 ± 1.7</td>
<td>11.5 ± 1.7</td>
</tr>
<tr>
<td>0.25</td>
<td>10.6 ± 1.9</td>
<td>9.1 ± 1.0</td>
<td>9.2 ± 1.6</td>
</tr>
<tr>
<td>0.5</td>
<td>11.4 ± 0.9</td>
<td>6.1 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8 ± 1.5</td>
</tr>
<tr>
<td>0.75</td>
<td>10.3 ± 1.9</td>
<td>5.4 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0 ± 3.6</td>
</tr>
<tr>
<td>1.0</td>
<td>10.8 ± 2.1</td>
<td>4.8 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.3 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
| 2.0               | 9.1 ± 1.7       | 3.3 ± 0.7<sup>b</sup> | 2.4 ± 0.5<sup>abcd</sup>

<sup>a</sup>Statistically different from sham control animals measured at the same time period.

<sup>b</sup>Statistically different from 0.25 Gy animals measured at the same time period.

<sup>c</sup>Statistically different from 0.50 Gy animals measured at the same time period.

<sup>d</sup>Statistically different from 0.75 Gy animals measured at the same time period.

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Fig. 1. Average and standard deviation of WBC count \( (\times 10^3 \) per \( \mu L \)) 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.

**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

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**Table 2. Absolute lymphocyte count data.**

<table>
<thead>
<tr>
<th>Exposure dose (Gy)</th>
<th>Day 0 ($\times 10^3$ per $\mu$L)</th>
<th>24 h ($\times 10^3$ per $\mu$L)</th>
<th>7 Day ($\times 10^3$ per $\mu$L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.9 ± 0.8</td>
<td>5.9 ± 0.8</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td>0.25</td>
<td>5.6 ± 1.2</td>
<td>4.4 ± 0.7^a</td>
<td>4.1 ± 0.8^a</td>
</tr>
<tr>
<td>0.5</td>
<td>6.3 ± 1.0</td>
<td>2.6 ± 0.3^b</td>
<td>3.1 ± 0.3^b</td>
</tr>
<tr>
<td>0.75</td>
<td>5.6 ± 0.3</td>
<td>2.0 ± 0.1^b</td>
<td>2.3 ± 0.4^b</td>
</tr>
<tr>
<td>1.0</td>
<td>5.7 ± 0.9</td>
<td>1.5 ± 0.4^bc</td>
<td>1.9 ± 0.4^bc</td>
</tr>
<tr>
<td>2.0</td>
<td>5.2 ± 1.0</td>
<td>0.9 ± 0.2^d,e</td>
<td>0.7 ± 0.1^d,e</td>
</tr>
</tbody>
</table>

^aStatistically different from sham control animals measured at the same time period.
^bStatistically different from 0.25 Gy animals measured at the same time period.
^cStatistically different from 0.50 Gy animals measured at the same time period.
^dStatistically different from 0.75 Gy animals measured at the same time period.
^eStatistically different from 1.0 Gy animals measured at the same time period.
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 Go¨ttingen 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 Go¨ttingen 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.

<table>
<thead>
<tr>
<th>Exposure dose (Gy)</th>
<th>Day 0 (%)</th>
<th>24 hr (%)</th>
<th>7 Day (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37.6 ± 5.6</td>
<td>37.7 ± 6.0</td>
<td>42.7 ± 9.3</td>
</tr>
<tr>
<td>0.25</td>
<td>39.9 ± 4.3</td>
<td>41.8 ± 2.6</td>
<td>49.2 ± 3.2</td>
</tr>
<tr>
<td>0.5</td>
<td>37.3 ± 6.4</td>
<td>45.9 ± 6.0</td>
<td>51.2 ± 6.8</td>
</tr>
<tr>
<td>0.75</td>
<td>36.8 ± 11.4</td>
<td>52.4 ± 10.1</td>
<td>59.7 ± 15.6</td>
</tr>
<tr>
<td>1.0</td>
<td>38.9 ± 8.5</td>
<td>59.8 ± 7.6$^{abc}$</td>
<td>55.7 ± 6.2</td>
</tr>
<tr>
<td>2.0</td>
<td>34.4 ± 6.4</td>
<td>68.1 ± 2.7$^{abcd}$</td>
<td>59.5 ± 6.1</td>
</tr>
</tbody>
</table>

$^a$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.

<table>
<thead>
<tr>
<th>Exposure dose (Gy)</th>
<th>Day 0 (× 10$^6$ per μL)</th>
<th>24 h (× 10$^6$ per μL)</th>
<th>7 Day (× 10$^6$ per μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.0 ± 0.5</td>
<td>8.3 ± 0.4</td>
<td>8.9 ± 0.6</td>
</tr>
<tr>
<td>0.25</td>
<td>9.0 ± 0.9</td>
<td>8.4 ± 1.0</td>
<td>8.6 ± 0.9</td>
</tr>
<tr>
<td>0.5</td>
<td>9.8 ± 0.5</td>
<td>8.9 ± 0.5</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>0.75</td>
<td>8.0 ± 0.5</td>
<td>7.7 ± 1.0</td>
<td>8.3 ± 0.3</td>
</tr>
<tr>
<td>1.0</td>
<td>8.7 ± 0.5</td>
<td>7.9 ± 0.6</td>
<td>8.4 ± 0.6</td>
</tr>
<tr>
<td>2.0</td>
<td>8.7 ± 0.5</td>
<td>8.6 ± 0.5</td>
<td>8.5 ± 0.7</td>
</tr>
</tbody>
</table>

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.
Fig. 4. Average and standard deviation of platelet count ($10^3 \text{L}^{-1}$) 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.
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.

Acknowledgments—This work was supported in part by Battelle Internal Research and Development funds and also with Federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, Department of Health and Human Services, under contract HHS0100201000010C. The authors wish to acknowledge and thank the dedicated animal care staff for their skilled handling of the animals.

REFERENCES


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

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

aStatistically different from sham control animals measured at the same time period.
bStatistically different from 0.25 Gy animals measured at the same time period.
cStatistically different from 0.50 Gy animals measured at the same time period.
dStatistically different from 0.75 Gy animals measured at the same time period.
eStatistically different from 1.0 Gy animals measured at the same time period.