Comparative Medicine
Copyright 2014
by the American Association for Laboratory Animal Science

Vol 64, No 6
December 2014
Pages 456–463

Original Research

Gastrointestinal Acute Radiation Syndrome in Göttingen Minipigs (*Sus Scrofa Domestica*)

Thomas B Elliott,[1] Nicolaas E Deutz,[6] Jatinder Gulani,[5] Amory Koch,[5] Cara H Olsen,[2] Christine Christensen,[3] Mark Chappell,[5] Mark H Whitnall,[2] and Maria Moroni[4,*]

In the absence of supportive care, exposing Göttingen minipigs to γ-radiation doses of less than 2 Gy achieves lethality due to hematopoietic acute radiation syndrome. Doses of 2 to 5 Gy are associated with an accelerated hematopoietic syndrome, characterized by villus blunting and fusion, the beginning of sepsis, and a mild transient reduction in plasma citrulline concentration. We exposed male Göttingen minipigs (age, 5 mo; weight, 9 to 11 kg) to γ-radiation doses of 5 to 12 Gy (total body; ^{60}Co, 0.6 Gy/min) to test whether these animals exhibit classic gastrointestinal acute radiation syndrome (GI-ARS). After exposure, the minipigs were monitored for 10 d by using clinical signs, CBC counts, and parameters associated with the development of the gastrointestinal syndrome. Göttingen minipigs exposed to γ radiation of 5 to 12 Gy demonstrate a dose-dependent occurrence of all parameters classically associated with acute GI-ARS. These results suggest that Göttingen minipigs may be a suitable model for studying GI-ARS after total body irradiation, but the use of supportive care to extend survival beyond 10 d is recommended. This study is the first step toward determining the feasibility of using Göttingen minipigs in testing the efficacy of candidate drugs for the treatment of GI-ARS after total body irradiation.

Abbreviation: GI-ARS, gastrointestinal acute radiation syndrome.

No drugs have been approved for the use of preventing gastrointestinal acute radiation syndrome (GI-ARS) in irradiated patients. The effect of the acute exposure of the GI tract to radiation traditionally has been associated with the inhibition of mitotic activity in intestinal crypts and the interrupted migration of GI epithelial cells from the crypts to the tips of the villi, leading to denudation of the intestinal mucosal barrier. The monolayer of epithelial cells lining the GI mucosa performs many functions of vital importance, including electrolyte transport, secretion of digestive fluids, absorption of nutrients, excretion of toxins, and providing a barrier to the luminal environment and commensal bacteria. The loss of intestinal barrier integrity results in the loss of nutrients, water, and electrolytes; increased permeability to bacteria and antigens; sepsis; inflammation; and organ dysfunction.

In humans, GI-ARS usually manifests at doses exceeding 5 to 6 Gy;[10] death occurs within 2 wk in the absence of treatment. Radiation-induced GI damage is accompanied by bone marrow suppression; the sequelae of GI-ARS and hematopoietic ARS partially overlap but do not necessarily develop concomitantly.[23] Characteristic of GI-ARS is the early onset of symptoms (nausea, vomiting, watery diarrhea, cramps) within a few hours, and the overt illness is marked by vomiting and diarrhea. Bacterial translocation from the intestinal tract, loss of crypts, shortening of villi, decreased citrulline levels, onset of abdominal pain, vomiting, and diarrhea are accepted markers of the GI syndrome.[22] Cardiovascular collapse, fluctuations in electrolyte concentrations, severe hemorrhage, and sepsis likely contribute to acute renal and multiorgan failure.[23]

Well-characterized animal models are required for efficacy testing of radiation countermeasures. So far, NHP are the only large animal model well-characterized in regard to the dose–survival relationship, symptoms, vital signs, and GI histology of GI-ARS.[11] In NHP, the $LD_{50/15}$ for GI-ARS in the presence of medical management including blood product transfusion is estimated to be 11.33 Gy.[11] Animals in the cited study[10] were characterized by shorter survival time compared with hematopoietic ARS, diarrhea, dehydration, and dose-dependent loss of body weight, intestinal crypts, and villi. In addition to the total body irradiation model, a partial-body irradiation model was established in NHP, by using 5% sparing of the bone marrow, to evaluate the long-term effects of radiation on the GI system and to study concomitant subsyndromes and organ injuries, including bone marrow and lungs.[10] Advantages of the partial body irradiation model are the ability to assess the development of various subsyndromes over time and the development of a polypharmacy approach targeting multiple organs. In dogs, acute GI-ARS was produced consistently by a single dose of 9.38 Gy to the abdomen.[20] In rodents, GI damage was induced by 8 to 15 Gy total body irradiation, depending upon the species and strain,[5,6,17,18] and was characterized by accelerated death, poor nutrient absorption, crypt depletion, intestinal epithe-

Received: 06 Jun 2014. Revision requested: 25 Jul 2014. Accepted: 14 Aug 2014.
[1]Combined Injury Program, [2]Department of Preventive Medicine and Biometrics, [3]Division of Comparative Pathology, [4]Radiation Countermeasures Program, and [5]Veterinary Sciences Department, Armed Forces Radiobiology Research Institute (AFRRI), Uniformed Services University of the Health Sciences, Bethesda, Maryland; [6]Department of Health & Kinesiology, Texas A&M University, College Station, Texas.
[*]Corresponding author. Email: maria.moroni@usuhs.edu

lial cells denudation, infections, diarrhea, dehydration, and weight loss. In mice, both acute and delayed effects of the GI syndrome have been identified; study endpoints evaluating medical countermeasures against GI-ARS have been proposed.[2,3] Acute effects were assessed at several levels of bone-marrow shielding and supportive care and were demonstrated by the presence of diarrhea, apoptosis, villus blunting, crypt depletion, loss of mucosal integrity, and ulcerations in the GI tract. Delayed effects included higher-than-normal levels of apoptotic and mitotic crypt cells, premature reductions in gut function, and the presence of adenomas.[2,3]

Additional large animal models are urgently needed to expedite drug testing and marketing under FDA regulations. Minipigs may be a potential alternative model for studying hematopoietic ARS,[15] but their high sensitivity to radiation prompts doubt regarding the feasibility of GI-ARS studies in this model. In the current study, we filled an important gap in development of minipigs as a model for ARS and addressed potential concerns regarding the feasibility of inducing classic GI-ARS in minipigs before they succumb to cardiovascular or respiratory complications. In previous publications, we documented hematopoietic ARS and accelerated hematopoietic ARS at higher radiation doses.[14,15] However, despite sporadic reports of specific types of radiation-induced injury to the GI tract of swine after total body irradiation, GI-ARS in that species has not been addressed comprehensively. To this end, we used a population of minipigs that was completely different from that in the previous studies and exposed the animals to much higher radiation doses than had been used previously. The subsequent results were markedly different from those of the previous reports, including loss of GI crypts and large decreases in circulating citrulline, both of which are important signs of GI-ARS. Our results demonstrate convincingly that GI-ARS occurs in swine and place this subsyndrome in the context of others studied comprehensively in the same strain. This achievement is an important milestone in the development of this much-needed large animal model of ARS, which promises to provide an alternative to NHP and dogs.

We show here that exposure of minipigs to γ irradiation in the range 5 to 12 Gy resulted in the development of signs typical of GI-ARS, and we discuss the importance of choosing the appropriate endpoint criteria for the different subsyndromes of ARS. Most minipigs died within 10 d, shifting the question from the feasibility of inducing GI-ARS in minipigs to the levels of supportive care appropriate for drug efficacy testing in this model.

Materials and Methods

Animals. Male **Göttingen** minipigs (n = 32; age, 4 to 5 mo; weight, 9 to 12 kg) were obtained from Marshall BioResources (North Rose, NY). All animal procedures were approved by the IACUC of the Armed Forces Radiobiology Research Institute, which is fully accredited by AAALAC. All procedures were consistent with the *Guide for the Care and Use of Laboratory Animals*,[16] AVMA euthanasia guidelines,[1] and the animal facility's standard operating procedures. Minipigs were fed twice daily with a commercial diet (8753 Minipig Teklad, Harlan Laboratories, Frederick, MD) according to their body weight and the supplier's recommendation. To facilitate blood sampling, a vascular access port was surgically implanted in each minipig 2 wk after arrival at our facility. Postoperative care consisted of carprofen (2 mg/kg PO; cherry flavored, Bio-Serve, Flemington, NJ) administered twice daily for 3 d and sulfamethoxazole–trimethoprim (60 mg PO; banana flavored, Bio-Serve, Flemington, NJ) administered once daily for 5 d. Minipigs were irradiated 3 wk after surgery. As a part of our basic characterization and in an attempt to understand the natural history of ARS in this model, supportive care was not provided. The vast majority of the animals were euthanized by day 10 after irradiation, according to preestablished endpoints.

Irradiation and blood sampling. Animals were irradiated with ^{60}Co γ photons, delivered bilaterally at 0.6 Gy/min as total body irradiation. The lowest dose was 5 Gy, according to the beginning of the appearance of bacterial translocation and death by day 10 after irradiation;[14] the highest dose was 12 Gy, which produced watery diarrhea within a few hours after irradiation. Experimental groups were: 5, 6, and 12 Gy, 2 pigs each; 7 and 11 Gy, 4 minipigs each; and 8, 9, and 10 Gy, 6 pigs each. All procedures for irradiation and blood sampling are described in detail elsewhere.[12] Briefly, the prescribed dose was delivered to the midline tissue at the widest cross-section of the abdomen, as measured with a caliper while the animal was lying in a sling. Real-time dosimetry was performed during irradiation with an ionization chamber, as a quality control check. The day of irradiation was considered day 0. Blood samples were obtained from the central port and immediately processed for CBC and differential counts, a clinical chemistry panel, and plasma citrulline.

Necropsy and histology. At the end of the study, all minipigs were anesthetized (6 to 8 mg/kg IM; Telazol) and then euthanized with an intravenous overdose of sodium pentobarbital (Euthasol, Virbac Animal Health, Fort Worth, TX). Euthanasia endpoints used were a combination of the following: weight loss, anorexia, diarrhea, vomiting, hypothermia (body temperature less than 36 °C) or hyperthermia (body temperature greater than 40 °C) in combination with neutropenia (absolute neutrophil count, less than 0.5×10^3 cells/μL), dyspnea with or without cyanosis, dehydration (increased capillary refill time or tacky mucus membranes or both). Full necropsies were performed on all animals, and a full complement of tissues was collected and immediately immersed in 10% neutral buffered formalin. Tissues were trimmed and processed according to standard protocols. Sections were prepared at a thickness of 5 to 6 μm, stained with hematoxylin and eosin, and evaluated by a board-certified veterinary pathologist. A crypt score was established to indicate the severity of tissue damage in the intestine, according to the presence and distribution of crypts at the base of the villi. The score was derived from the comparison of irradiated sections with normal intestinal villi from sham-irradiated minipigs. Sections were scored as: 0, normal (6 crypts at the base of each villus); 1, minimal crypt loss (5 crypts per villus); 2, mild crypt loss (4 crypts per villus); 3, moderate crypt loss (fewer than 4 crypts per villus, evenly distributed circumferentially); 4, marked crypt loss (fewer than 3 crypts per villus, multifocal areas with no crypts circumferentially), and 5, severe crypt loss (large areas with no crypts and just a few crypts circumferentially).

Experimental endpoints. Primary study endpoints were survival at 10 d, mean survival time, signs, CBC counts, thrombocytopenia and febrile neutropenia, and time to onset; secondary endpoints were diarrhea, vomiting, major clinical signs, hematologic parameters, citrulline concentration, bacterial translocation, histology of the intestine, and clinical chemistry assays to evaluate blood glucose, kidney and liver function, and electrolyte and fluid balance.

Microbiology. For isolation and identification of bacteria from moribund minipigs, blood samples were collected aseptically and prior to euthanasia from either the vascular access port in unanesthetized minipigs or the heart ventricle in deeply anesthetized an-

imals. After blood sampling, moribund minipigs were euthanized as described. Immediately after euthanasia, specimens of liver and spleen tissues were removed aseptically. Facultative bacteria were isolated from these tissues according to routine standard microbiologic procedures as previously described.[14] Briefly, specimens of peripheral blood were inoculated aseptically and directly through a fresh, sterile needle and into a blood-culture bottle, which contained liquid culture medium and beads that inactivate antimicrobial agents (BacTec Peds Plus/F Culture Vial, Becton Dickinson, Sparks, MD). Blood cultures were incubated for 7 d at 35 °C. They were checked daily for turbidity. When turbid, 2 or 3 drops of culture fluid were transferred by sterile syringe and needle to culture media, as described for the primary culture of tissues. All blood cultures that did not demonstrate turbidity after 7 d were subcultured on day 7 to confirm sterility.

Sterile polyester swabs were used to penetrate specimens of liver and spleen collected after euthanasia. The swabs were inoculated and distributed by 4-dilution streaking with a sterilized bacteriological loop onto 5% sheep blood in Columbia agar, colistin–nalidixic acid in sheep blood agar, and xylose–lysine–desoxycholate agar (Remel, Fisher Scientific, Pittsburgh, PA). Samples in sheep blood agar with or without colistin–nalidixic acid were incubated for 18 to 24 h at 35 °C in 5% CO_2; those in xylose–lysine–desoxycholate agar were incubated at 35 °C. Sheep blood agar is an enriched, nonselective medium; colistin–nalidixic acid in sheep blood agar is selective for gram-positive bacteria; and xylose–lysine–desoxycholate agar is selective for gram-negative bacteria. Culture media without observable growth after 24 h were incubated for an additional 24 h. Colony morphologies were observed and recorded. Individual colonies were differentiated and selected for subculture after Gram staining. Pure subcultures were identified by using an automated system (Vitek 2 Compact, bioMérieux, Durham, NC).

Plasma citrulline. Plasma amino acid concentrations were measured as described previously.[14]

Statistical analysis. Basic descriptive analyses and 2-sided Student *t* tests completed by using Microsoft Excel (Redmond, WA). Results are expressed as mean ± SE or mean ± 1 SD. The level of significance was set at a P value less than 0.05. The statistical package within Prism (version 6.03, GraphPad, San Diego, CA) was used for data analysis. Linear regression analysis and Pearson correlation were used to calculate the strength and significance of the relations between the plasma citrulline concentrations, radiation dose, and crypt score.

Results

Natural history. ***Signs.*** Mean survival time of minipigs that underwent total body γ irradiation was dose-dependent (Table 1). Animals displayed recurrent periods of slow activity and recumbency, decreased appetite, hunched posture, anorexia, and lameness. At times, the heart rate was irregular and weak. Epistaxis and hematuria were noted occasionally. The severity of clinical signs was dose-dependent.

In minipigs irradiated with 5 to 7 Gy, loose stools began on day 6 to 7 and continued thereafter; moderate to severe abdominal distention was evident beginning 2 to 3 d after irradiation. Bilious vomiting was sporadic, beginning on day 8 to 9. Shallow breathing with wheezing became evident in most animals at approximately day 6 to 7 and lasted until the animal became moribund. Labored breathing and cyanosis appeared on the day of euthanasia.

Table 1. Dose–response relationship in average survival time of irradiated minipigs (n = 32 total)

Dose (Gy)	No. of minipigs	Survival time (d)	1 SD	Minimum	Maximum
5	2	8; 10[a]	na	na	na
6	2	9; 9[a]	na	na	na
7	4	7[b]	1	7	8
8	6	8[b]	1	6	10
9	6	7[b]	1	6	8
10	6	6[b]	1	4	6
11	4	6[b]	1	5	6
12	2	5; 5[a]	na	na	na

na, not applicable
Data are reported as [a]individual values or [b]group means.

In minipigs irradiated with 9 to 12 Gy, dehydration (inferred from tenting of the skin and dry mucous membranes) and poor perfusion (inferred from cold extremities) began on day 1 after irradiation and continued thereafter and were more severe than those observed at lower doses. Transient retching, abdominal cramping (as evidenced by hunched posture), and vomiting occurred shortly after irradiation. Vomiting initially subsided but began again on day 3 or thereafter. At these radiation doses, increased bowel movements were apparent within hours after irradiation; watery diarrhea was noticed terminally (day 5 to 8). Tissue perfusion was reduced for all animals, given the appearance of mucous membranes and cold extremities. Transient cardiac arrhythmia was present, usually starting 1 to 2 d after irradiation and in some cases persisting for several days. Animals were febrile (temperature greater than 39 °C) as early as 3 h after irradiation; fever was transient and lasted less than 24 h. In addition, minipigs developed fever when they became moribund. For most minipigs given 9 to 12 Gy, respiratory distress occurred only when they became moribund, unlike the situation in animals irradiated at lower doses, which experienced respiratory distress for more than 24 h.

Hematologic and clinical chemistry parameters. Hematologic analysis of the GI subsyndrome indicated that the decline in blood cell numbers was dose-dependent and started soon after exposure (Figure 1). Grade 3 neutropenia (absolute neutrophil count, less than 500 μL^{-1}) was reached by day 7 in minipigs given 5 to 9 Gy and by day 3 in animals given 10 to 12 Gy. WBC loss paralleled that of the neutrophil count.

The incidence and time course of febrile neutropenia were dose-dependent also, with 65% (21 of 32) of the animals displaying febrile neutropenia, which began 7.6 ± 0.6 d after irradiation. Thrombocytopenia (fewer than 20,000 μL^{-1}) typically occurred by day 10 after 5 to 9 Gy and by day 7 after 10 or 11 Gy. Animals given 12 Gy did not exhibit thrombocytopenia because they became morbid and moribund before platelet numbers began to decrease. RBC declines were gradual.

To evaluate effects on specific organ function at different doses of irradiation, assays were performed to evaluate kidney function (BUN and creatinine), liver function (GGT, lactate dehydrogenase, ALT, ALP, AST, albumin, globulins, and total protein), and pancreatic function (amylase and lipase). Values representing the averages for all exposures regardless of dose are reported for selected parameters in Table 2. Irradiation did not appear to affect pancreatic function, whereas indicators of kidney function were

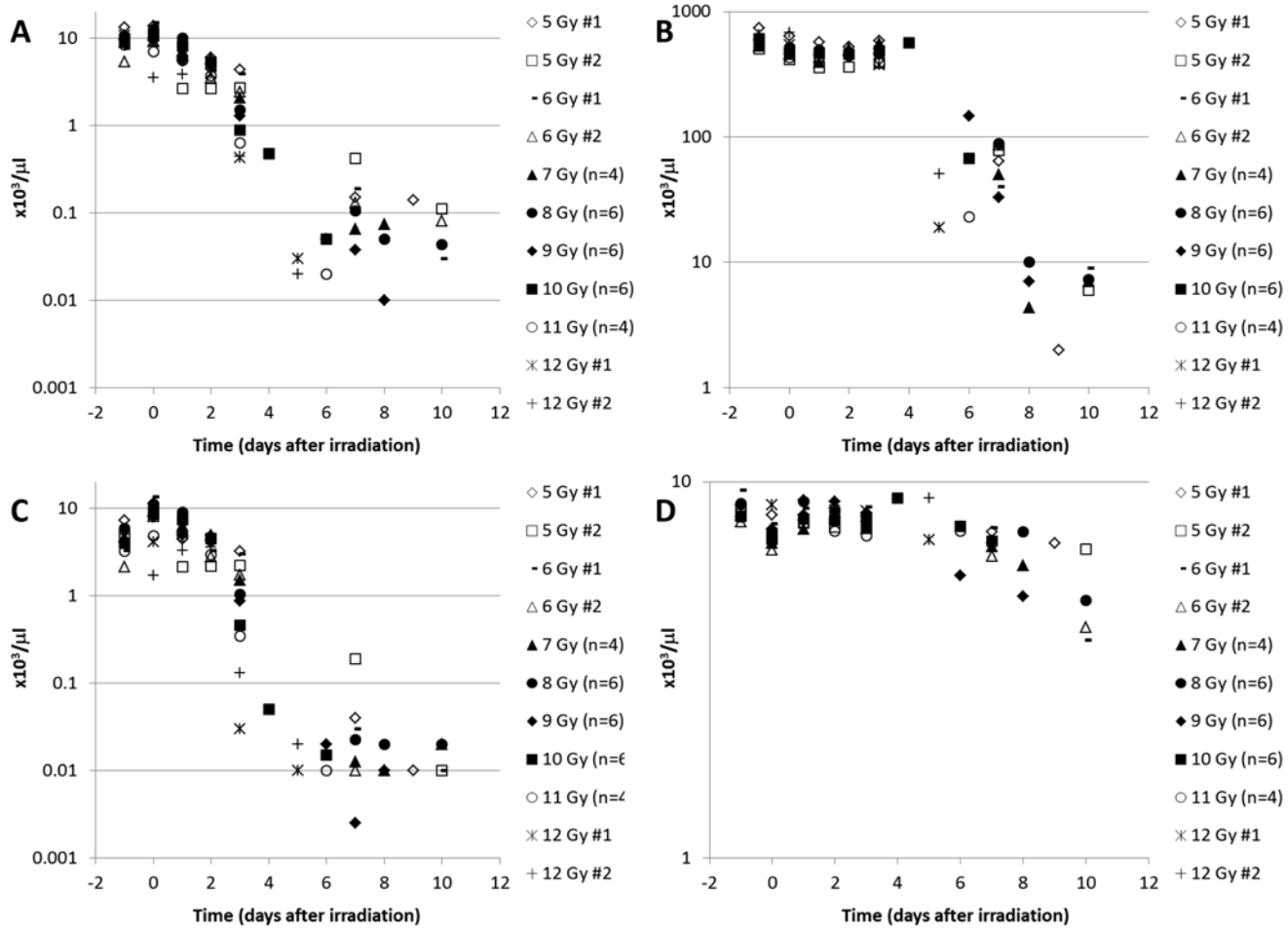

Figure 1. Patterns of mean blood counts in minipigs irradiated with 5 to 12 Gy. (A) WBC. (B) Platelets. (C) Neutrophils. (D) RBC.

Table 2. Selected clinical chemistry parameters (mean ± 1 SD) before irradiation (baseline) and at necropsy

	Baseline	At necropsy	*P*
BUN (mg/dL)	5.7 ± 1.0	19.7 ± 15.5	0.05
Creatinine (mg/dL)	0.9 ± 0.1	1.2 ± 0.2	0.03
GGT (U/L)	107.8 ± 30.8	40.3 ± 5.7	0.000
LDH (U/L)	1166.4 ± 120.1	823.1 ± 174.2	0.001
Albumin (g/dL)	4.1 ± 0.2	3.6 ± 0.2	<0.001
Globulin (g/dL)	2.3 ± 0.1	2.9 ± 0.2	<0.001
Albumin:globulin	1.8 ± 0.1	1.2 ± 0.1	<0.001
ALT (U/L)	23.8 ± 14.5	25.7 ± 6.1	0.67
ALP (U/L)	162.4 ± 17.3	163.5 ± 49.3	0.96
AST (U/L)	39.3 ± 10.7	68.0 ± 44.4	0.14
Protein (g/dL)	6.3 ± 0.2	6.5 ± 0.3	0.25
Amylase (U/L)	600.3 ± 130.8	873.1 ± 989.1	0.49
Lipase (U/L)	96.6 ± 122.2	131.1 ± 190.2	0.67

consistently altered at necropsy; urine specific gravity was not measured. Regarding liver enzymes and parameters, GGT, lactate dehydrogenase, and albumin decreased significantly ($P \leq 0.001$); globulins increased ($P < 0.001$); and ALT, ALP, AST, and total protein did not change. The albumin:globulin ratio was decreased dramatically at necropsy, reflecting changes in both albumin and globulin plasma levels, again suggesting possible dysfunction in both kidney and liver.

Microbiology. Sepsis, as indicated by isolation of bacteria from blood and tissues (spleen and liver), was evident in 29 of the 31 moribund, irradiated minipigs tested. Doses of radiation greater than 5 Gy, particularly greater than 9 Gy, were sufficient to induce a well-developed systemic infection of tissues, caused by 11 gram-positive species and 4 gram-negative species (Table 3). The average number of bacterial species translocated from the gastrointestinal tract appeared to increase with radiation dose.

Histology and plasma citrulline. Variable amounts of pulmonary hemorrhage and edema were evident in most minipigs given 10 Gy or less. With doses exceeding 10 Gy, pulmonary edema was minimal to moderate and occurred consistently without hemorrhage. Bacterial lung infection was observed in most animals irradiated with 6 to 12 Gy. Doses between 5 and 8 Gy produced mild to moderate blunting of intestinal villi. At exposures of 9 Gy and greater, intestinal villi were moderately to markedly blunted and fused. The loss of villi and crypts

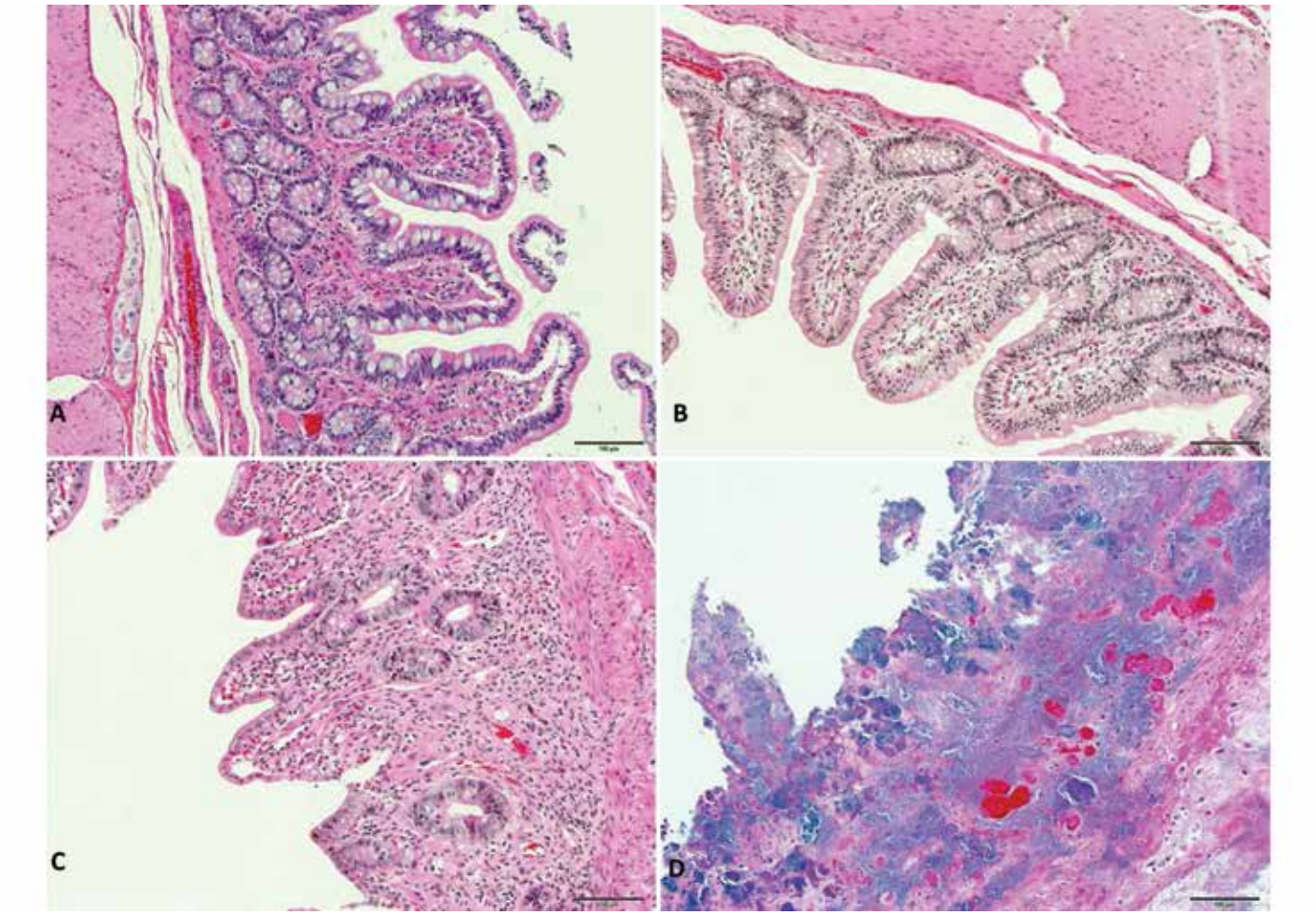

Figure 2. Histology of jejunum of minipigs irradiated with ^{60}Co γ-radiation. (A) Jejunum, 5 Gy: mild intestinal villus blunting and fusion with essentially normal crypts. (B) Jejunum, 9 Gy: moderate intestinal villus blunting and fusion with moderate crypt loss. (C) Jejunum, 11 Gy: marked intestinal villus blunting and fusion with marked crypt loss. (D) Jejunum, 12 Gy: severe loss of intestinal villi and crypts with necrosis and abundant intralesional bacteria. Magnification, 20×.

Table 3. Bacterial species isolated from blood or tissues of irradiated Göttingen minipigs

Dose (Gy)	Bacterial translocation?	No. of septic pigs/total no. irradiated (%)	Average no. of bacterial species per pig (minimum/maximum)
5.0	Yes/ No	1/2 (50%)	1.5 (0/3)
6.0	Yes	2/2 (100%)	1.5 (1/2)
7.0	Yes	4/4 (100%)	2.0 (2/3)
8.0	Yes	6/6 (100%)	2.3 (1/4)
9.0	Yes / No	5/6 (83.33%)	2.0 (0/4)
10.0	Yes	6/6 (100%)	2.2 (1/5)
11.0[a]	Yes	3/3 (100%)	3.3 (3/4)
12.0	Yes	2/2 (100%)	3.0 (2/4)

Gram-positive strains included *Staphylococcus chromogenes*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Streptococcus alactolyticus*, *Streptococcus dysgalactiae* ssp. *equisimilis*, *Streptococcus infantarius* ssp. *coli*, *Streptococcus mitis* or *Streptococcus oralis*, *Enterococcus cecorum*, *Enterococcus columbae*, *Enterococcus faecalis*, and *Actinomyces naeslundii*.
Gram-negative strains included *Escherichia coli*, *Klebsiella pneumoniae* ssp. *pneumoniae*, *Pasteurella pneumotropica*, and *Pseudomonas aeruginosa*.
Yeast strain was *Candida tropicalis*.
[a]Samples unavailable for 1 of the 4 pigs irradiated at this dose.

appeared directly proportional to radiation dose (Figure 2). At 12 Gy, consistent and severe loss of intestinal crypts, severe blunting, fusion and loss of intestinal villi, necrosis, and intralesional bacteria occurred.

In pigs given doses of 6 Gy or greater, plasma citrulline concentrations (μM) declined continuously (Figure 3 A). At doses greater than 9 Gy, the decline in citrulline concentration relative to preirradiation levels was significant ($P < 0.05$) starting at day 3. After doses of 10 Gy and greater, a significant ($P < 0.05$) decline in citrulline levels occurred beginning on day 2 after irradiation. The correlation between radiation dose and crypt score is shown in Figure 3 B. Higher radiation doses were associated with higher crypt scores at necropsy ($r = 0.725$, $P < 0.0001$), confirming a dose-dependence between radiation dose and the severity of GI damage in minipigs.

A significant negative correlation was established between radiation dose and plasma citrulline concentration on days 2, 3, and 7 after irradiation (day 2: $r = -0.408$, $P = 0.0427$; day 3: $r = -0.716$, $P < 0.0001$; day 7: $r = -0.833$, $P < 0.0001$; Figure 4 A) as well as between crypt score at necropsy and plasma citrulline measured on day 3 ($r = -0.545$, $P = 0.0072$) and day 7 ($r = -0.558$, $P = 0.0037$) but not day 2 ($r = -0.297$, $P = 0.158$; Figure 4 B).

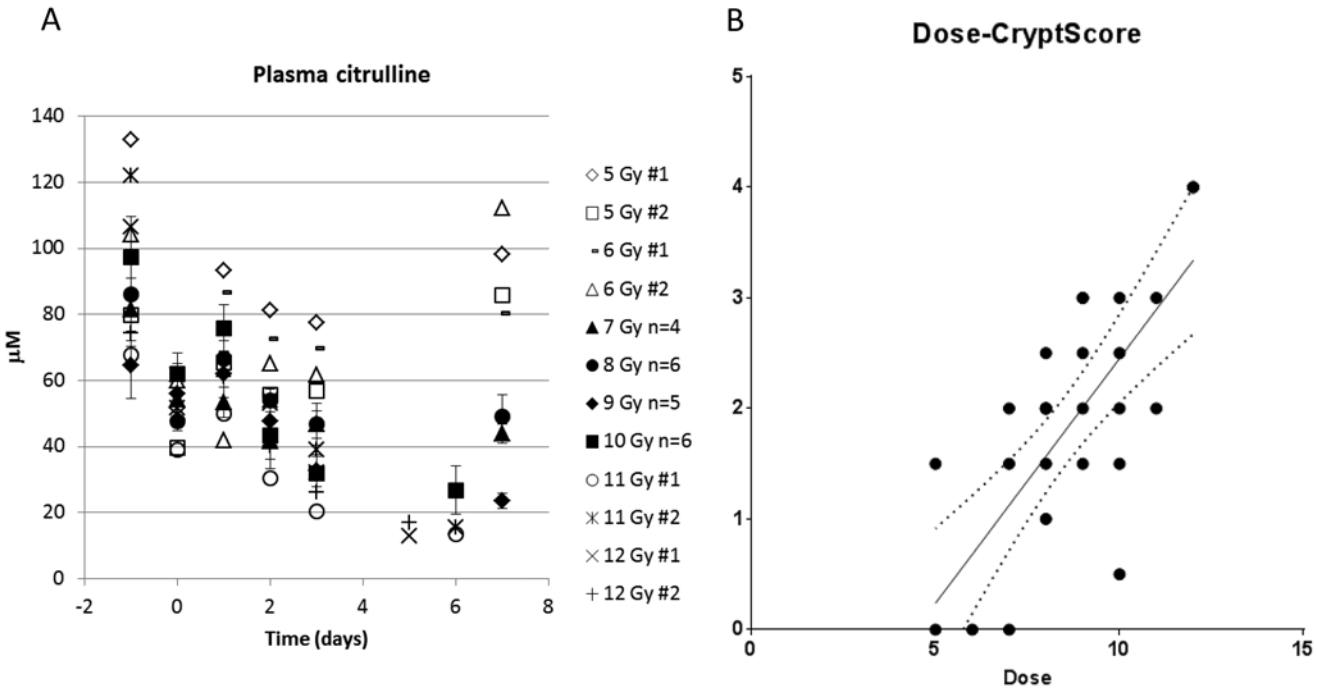

Figure 3. Mean citrulline concentration in plasma of minipigs given 5 to 12 Gy ^{60}Co γ-radiation and relationship between dose and crypt score at necropsy. (A) Dose- and time-dependent decline in plasma citrulline levels. After doses of 10 Gy or more, citrulline concentrations were significantly ($P < 0.05$) decreased as early as 2 d after irradiation and did not show signs of recovery within 7 d after irradiation ($P < 0.01$). (B) Crypt score at necropsy, which describes severity of crypt loss, was positively correlated ($P < 0.0001$) with dose of radiation.

Discussion

A comprehensive study of the effect of total body irradiation on the induction of GI-ARS in minipigs and a description of the progression of the disease have not been reported previously. Studies by other authors include the characterization of GI-ARS in Göttingen minipigs surgically modified with an ileocutaneous anastomosis and irradiated locally to the abdomen, and the effect of irradiation on the energy metabolism in small intestine of Tibet minipigs.[19,22] The first study cited[19] demonstrates the utility of minipigs for studying radiation-induced morphologic, histologic, and functional changes to the GI tract and supports the use of citrulline as a potential marker for radiation-induced intestinal damage. Our results are consistent with these previous findings,[19] in terms of loss of crypts and villi and decline in plasma citrulline, thereby supporting the validity of minipigs as a model for GI-ARS. The other previous study[22] used total body irradiation, with euthanasia of minipigs 24 h after irradiation for tissue collection and analysis of early response of energy metabolism. The aim of our current study was to evaluate whether Göttingen minipigs develop GI-ARS after total body irradiation before succumbing to the cardiovascular complications observed at doses leading to hematopoietic ARS. Despite the high sensitivity of minipigs to radiation, our study showed that it is possible to induce GI-ARS in Göttingen minipigs and that their signs resemble those in humans.

A case of GI-ARS occurred in China in 2008,[7] after an accidental dose of 14.5 Gy from a ^{60}Co source. The patient displayed vomiting, fever, and skin erythema, and blood counts declined shortly after exposure. The patient was hospitalized and received full supportive care, including stem cell transplantation. The patient developed sustained diarrhea starting at day 4, lung infection, and signs of systemic inflammatory response syndrome. Blood cell counts continued to decrease, and damage to the GI tract became evident 40 d after autologous stem cell transplantation. The patient eventually, in the hospital from multiorgan failure associated with GI damage. At autopsy, copious blood was found in the abdominal cavity, in combination with extensive necrosis and sloughing of the mucosa of the small intestine and colon.[7]

Irradiated minipigs demonstrated most of the early signs displayed by patients with GI-ARS. The major difference is that intensive supportive care and transplantation are likely to alter some of these signs and to extend the survival of human patients, thus providing the opportunity for the development of delayed and concurrent subsyndromes. Minipigs irradiated in the dose range of 5 to 12 Gy displayed dose-dependent vomiting, diarrhea, bacterial translocation, and declines in plasma citrulline levels. Retching and abdominal cramps occurred after irradiation with doses of less than 10 Gy, and vomiting developed after the receipt 11 or 12 Gy.

Minipigs displayed recurrent periods of reduced activity and recumbence, decreased appetite, hunched posture, anorexia, and lameness. Because of the transient and recurrent nature of the signs, one of our major challenges for the study was to find reliable endpoints to predict mortality. Minipigs given doses sufficient to cause hematopoietic ARS[13]exhibited a very characteristic period of 36 to 48 h of lameness and anorexia before meeting study endpoint criteria; these signs proved to be reliable surrogate markers for mortality and indicators for reducing animal pain and distress or for therapy. In the current study, after receiving 5 to 12 Gy, animals were euthanized mainly because they exhibited a combination of anorexia, vomiting, lethargy, and respiratory distress. However, duration of these signs varied with the dose

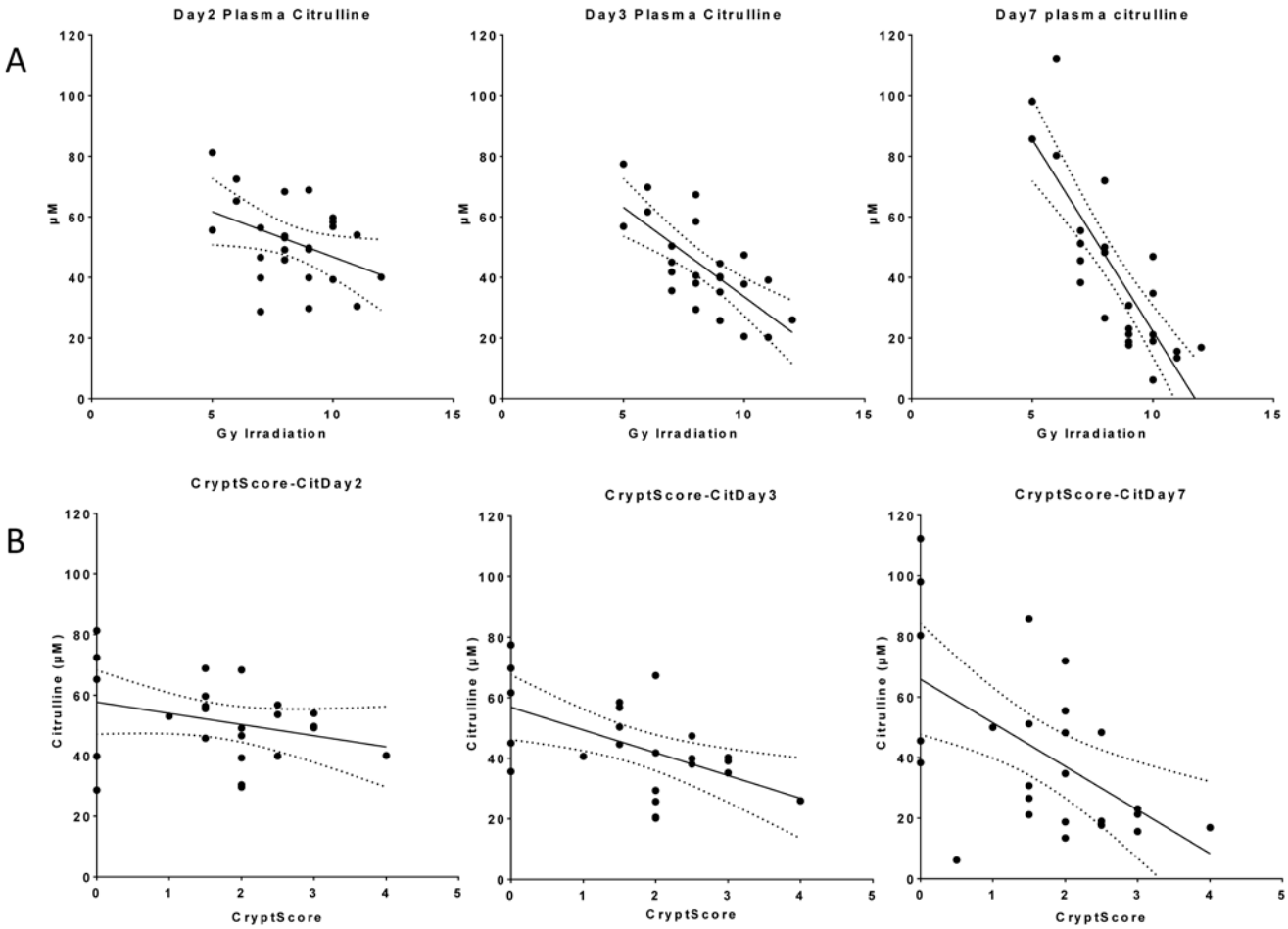

Figure 4. Dose-dependent loss of citrulline over time and relationship between plasma citrulline concentration and crypt score at necropsy in minipigs after irradiation. Top panels show the relationship between radiation dose and citrulline levels on days 2 (left), 3 (center), and 7 (right) after irradiation. Bottom panels show the relationship between crypt score at necropsy and plasma citrulline level on days 2 (left), 3 (center), and 7 (right) after irradiation.

delivered, likely reflecting the sensitivity of the organ (that is, lungs compared with GI) primarily involved in the cause of morbidity, and made it challenging to reliably apply preestablished endpoints. So, although respiratory distress lasted 2 to 3 d after doses at or below 9 Gy, the consequences of GI damage appeared to predominate at higher doses, and respiratory patterns suggestive of lung complications did not have time to develop fully.

Microscopically, the extent and severity of intestinal crypt loss and villus blunting and fusion were essentially directly proportional to radiation dose, with mild to moderate changes after doses between 5 and 8 Gy and marked to severe changes after doses of 9 Gy or more. Doses of radiation greater than 9 Gy were sufficient to induce consistently a well-developed systemic polymicrobial sepsis and infection of tissues in minipigs, whereas indications of early or developing infection were detected in minipigs receiving doses of 5 to 9 Gy. Lung infection was a common characteristic among most minipigs.

Circulating citrulline concentrations have been established as a reliable biomarker for the clinical assessment of ionizing radiation damage and have routinely been used as a marker for the gastrointestinal syndrome. The use of plasma citrulline as a proxy for gastrointestinal function has been shown to be effective in patients with short-bowel syndrome[4] as well as after irradiation and chemotherapy. In the past, mouse models have been used to study the effects of radiation on the gastrointestinal tract[21,22] and have provided a very good correlation between plasma citrulline and damage to the intestines. Our data in minipigs show that radiation damage to gastrointestinal integrity was dose-dependent and that there was a significant correlation between citrulline levels and radiation dose starting at day 3 after irradiation. Because production of citrulline depends almost exclusively on functional intestinal enterocytes, we measured the correlation between radiation dose and crypt score and found a positive relation between the 2 parameters. Importantly, similar to humans and other animal models, citrulline levels predicted the severity of GI damage in minipigs.

This exploratory study is limited by the small number of minipigs used per radiation dose and by the lack of knowledge regarding the kinetics of intestinal epithelial cell turnover. Extending survival times beyond 8 to 10 d through supportive care is expected to be a necessary requirement to allow for sufficient time to assess crypt epithelial cell transit time and countermeasure efficacy.[11] Otherwise, targeted irradiation of the GI may be sufficient for evaluating countermeasure efficacy, as has been proposed for

dogs.[8] Nevertheless, the signs observed and data collected in the current study suggest that Göttingen minipigs are a suitable animal model for comparison to human GI-ARS.

Acknowledgments

Special thanks are due to AFRRI's ^{60}Co facility and the Veterinary Sciences Department staff for their dedication to the project and superb animal care. The findings and views expressed in this report are those of the authors and do not represent official policy of the Armed Forces Radiobiology Research Institute, the Uniformed Services University of the Health Sciences, or the U.S. Department of Defense.

References

1. **American Veterinary Medical Association.** 2013. AVMA guidelines for the euthanasia of animals: 2013 ed. Schaumberg (IL): American Veterinary Medical Association.
2. **Booth C, Tudor G, Tonge N, Shea-Donohue T, MacVittie TJ.** 2012b. Evidence of delayed gastrointestinal syndrome in high-dose irradiated mice. Health Phys **103:**400–410.
3. **Booth C, Tudor G, Tudor J, Katz BP, MacVittie T.** 2012a. The acute gastrointestinal syndrome in high-dose irradiated mice. Health Phys **103:**383–399.
4. **Crenn P, Coudray-Lucas C, Thuillier F, Cynober L, Messing B.** 2000. Post-absorptive plasma citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans. Gastroenterology **119:**1496–1505.
5. **Driák D, Osterreicher J, Vávrová J, Reháková Z, Vilasová Z.** 2008. Morphological changes of rat jejunum after whole body gamma-irradiation and their impact in biodosimetry. Physiol Res **57:**475–479.
6. **Geraci JP, Dunston SG, Jackson KL, Mariano MS, Holeski C, Eaton DL.** 1987. Bile loss in the acute intestinal radiation syndrome in rats. Radiat Res **109:**47–57.
7. **Guo M, Dong Z, Qiao J, Yu C, Sun Q, Hu K, Liu G, Wei L, Yao B, Man Q, Sun X, Liu Z, Song Z, Yu C, Chen Y, Luo Q, Liu S, Ai HS.** 2014. Severe acute radiation syndrome: treatment of a lethally ^{60}Co-source irradiated accident victim in China with HLA-mismatched peripheral blood stem cell transplantation and mesenchymal stem cells. J Radiat Res (Tokyo) **55:**205–209.
8. **Lutgens LC, Blijlevens NM, Deutz NE, Donnelly JP, Lambin P, de Pauw BE.** 2005. Monitoring myeloablative therapy-induced small bowel toxicity by serum citrulline concentration: a comparison with sugar permeability tests. Cancer **103:**191–199.
9. **Lutgens LC, Deutz NE, Gueulette J, Cleutjens JP, Berger MP, Wouters BG, von Meyenfeldt MF, Lambin P**. 2003. Citrulline: a physiologic marker enabling quantitation and monitoring of epithelial radiation-induced small bowel damage. Int J Radiat Oncol Biol Phys **57:**1067–1074.
10. **MacVittie TJ, Bennett A, Booth C, Garofalo M, Tudor G, Ward A, Shea-Donohue T, Gelfond D, McFarland E, Jackson W 3rd, Lu W, Farese AM.** 2012. The prolonged gastrointestinal syndrome in rhesus macaques: the relationship between gastrointestinal, hematopoietic, and delayed multi-organ sequelae following acute, potentially lethal, partial-body irradiation. Health Phys **103:**427–453.
11. **MacVittie TJ, Farese AM, Bennett A, Gelfond D, Shea-Donohue T, Tudor G, Booth C, McFarland E, Jackson W 3rd.** 2012. The acute gastrointestinal subsyndrome of the acute radiation syndrome: a rhesus macaque model. Health Phys **103:**411–426.
12. **Military Medical Operations, Armed Forces Radiobiology Research Institute Bethesda.** 2009. Medical management of radiological casualties, 3rd ed. Bethesda (MD): Armed Forces Radiobiology Research Institute.
13. **Moroni M, Coolbaugh TV, Lombardini E, Mitchell JM, Moccia KD, Shelton LJ, Nagy V, Whitnall MH.** 2011. Hematopoietic radiation syndrome in the Göttingen minipig. Radiat Res **176:**89–101.
14. **Moroni M, Elliott TB, Deutz NE, Olsen CH, Owens R, Christensen C, Lombardini ED, Whitnall MH.** 2014. Accelerated hematopoietic syndrome after radiation doses bridging hematopoietic (H-ARS) and gastrointestinal (GI-ARS) acute radiation syndrome: early hematological changes and systemic inflammatory response syndrome in minipig. Int J Radiat Biol **90:**363–372.
15. **Moroni M, Lombardini E, Salber R, Kazemzedeh M, Nagy V, Olsen C, Whitnall MH.** 2011. Hematological changes as prognostic indicators of survival: similarities between Göttingen minipigs, humans, and other large animal models. PLoS ONE **6:**e25210.
16. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
17. **Potten CS.** 1990. A comprehensive study of the radiobiological response of the murine (BDF1) small intestine. Int J Radiat Biol **58:**925–973.
18. **Saha S, Bhanja P, Liu L, Alfieri AA, Yu D, Kandimalla ER, Agrawal S, Guha C.** 2012. TLR9 agonist protects mice from radiation-induced gastrointestinal syndrome. PLoS ONE **7:**e29357.
19. **Shim S, Jang WS, Lee SJ, Jin S, Kim J, Lee SS, Bang HY, Jeon BS, Park S.** 2014. Development of a new minipig model to study radiation-induced gastrointestinal syndrome and its application in clinical research. Radiat Res **181:**387–395.
20. **Summers RW, Flatt AJ, Prihoda MJ, Mitros FA.** 1987. Effect of irradiation on morphology and motility of canine small intestine. Dig Dis Sci **32:**1402–1410.
21. **Van Vliet MJ, Tissing WJE, Rings EHHM, Koetse HA, Stellaard F, Kamps WA, de Bont ES.** 2009. Citrulline as a marker for chemotherapy induced mucosal barrier injury in pediatric patients. Pediatr Blood Cancer **53:**1188–1194.
22. **Wang YJ, Liu W, Chen C, Yan LM, Song J, Guo KY, Wang G, Wu QH, Gu WW.** 2013. Irradiation induced injury reduces energy metabolism in small intestine of Tibet minipigs. PLoS ONE **8:**e58970.
23. **Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, Tsu H, Confer DL, Coleman CN, Seed T, Lowry P, Armitage JO, Dainiak N.** 2004. Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. Ann Intern Med **140:**1037–1051.