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Vascular Access Port Implantation and Serial Blood Sampling in a Gottingen Minipig (*Sus scrofa domestica*) Model of Acute Radiation Injury

Maria Moroni,[1,*] Thea V Coolbaugh,[1] Jennifer M Mitchell,[2] Eric Lombardini,[2] Krinon D Moccia,[2] Larry J Shelton,[2] Vitaly Nagy,[3] and Mark H Whitnall[1]

Threats of nuclear and other radiologic exposures have been increasing, but no countermeasure for acute radiation syndrome has been approved by regulatory authorities. Because of their similarity to humans in regard to physiology and anatomy, we are characterizing Gottingen minipigs as a model to aid the development of radiation countermeasures. Irradiated minipigs exhibit immunosuppression, severe thrombocytopenia, vascular leakage, and acute inflammation. These complications render serial acquisition of blood samples problematic. Vascular access ports (VAP) facilitate serial sampling, but their use often is complicated by infections and fibrin deposition. We demonstrate here the successful use of VAP for multiple blood samplings in irradiated minipigs. Device design and limited postoperative prophylactic antimicrobial therapy before irradiation were key to obtaining serial sampling, reducing swelling, and eliminating infection and skin necrosis at the implantation site. Modifications of previous protocols included the use of polydioxanone sutures instead of silk; eliminating chronic port access; single-use, sterile, antireflux prefilled syringes for flushing; strict aseptic weekly maintenance of the device, and acclimating animals to reduce stress. VAP remained functional in 19 of 20 irradiated animals for as long as 3 mo. The remaining VAP failed due to a small leak in the catheter, leading to clot formation. VAP-related sepsis occurred in 2 minipigs. Blood sampling did not cause detectable stress in nonanesthetized sham-irradiated animals, according to leukograms and clinical signs.

Abbreviations: ARS, acute radiation syndrome; VAP, vascular access port.

Advanced drug development for radiation injuries has been hampered by the lack of sufficient number and availability of animal models that can be used for research. The only options at present for long-lived, nonrodent animal models that have been well-characterized in terms of radiation injury for advanced drug development are nonhuman primates and canines.[2] Swine currently are being suggested as a potential model for validation of mitigators and therapeutic drugs for acute radiation syndrome (ARS).[2] However, a systematic study of ARS in swine has not been reported.

Swine share many similarities with humans in terms of anatomy, physiology, and drug metabolism.[30] Minipigs have been used to evaluate drug pharmacology and toxicology [14,17,31] and guidelines from the US Food and Drug Administration mention minipigs as an acceptable species for safety evaluation of drugs.[33] Because of the availability of control background data, small size, defined microbiologic and genetic backgrounds, and docility, we selected Gottingen minipigs as a potential model for ARS studies.

ARS causes severe damage to the hematopoietic system, severe immunosuppression, thrombocytopenia, neutropenia, and acute inflammation, leading to possible death.[4] Most studies of ARS in large animal models have concentrated on the numbers of circulating blood elements (lymphocytes, neutrophils, and platelets), monitored at frequent intervals, and survival, for assessment of radiation doses. Monitoring assays for therapeutic medical management of radiation victims are based on lymphocyte cytologic tests and CBC with differential blood cell counts.[35] Under normal clinical conditions, repeated blood sampling is associated with complications including tissue reactions, prolonged bleeding, and risk of infections.[32] These issues are exacerbated in irradiated animals, in which infection, hemorrhage, and capillary fragility[8] are the main factors in death. Difficulty in successful acquisition of serial blood samples can thus hinder completion of an important component of the experimental design for in vivo studies of ARS. A potential solution to these problems is the use of subcutaneous vascular access ports (VAP), which provide easy access to vessels. VAP consist of a port surgically infiltrated within the subcutis, firmly sutured in place and accessible through the skin with a needle puncture, and connected to a catheter implanted subcutaneously that feeds into a peripheral vein. The catheter does not exit through the skin, and the risks of infection, tissue damage, and morbidity are reduced.[7] Nonetheless, the shape, material, surgical technique, and postoperative maintenance of VAP affect the success of their usage.[29]

Subcutaneous VAP have been used in clinical settings as an alternative to externalized catheters to reduce risks of infection, inflammation, hematoma, and prolonged bleeding—difficulties associated with repeated blood sampling.[27] However, the use of VAP can be associated with complications, including sepsis

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[1]Radiation Countermeasures Program, Scientific Research Department, and Departments of [2]Veterinary Sciences and [3]Radiation Sources, Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, Maryland.
[*]Corresponding author. Email: moroni@afrri.usuhs.mil

and thrombosis, which can increase morbidity and mortality and alter the drug administration regimen.[39] Rates of long-term complications (for example, sepsis, thrombosis, occlusion) of port and catheter use have been reported and vary greatly, between 0.6% and 27%.[6,24,38] Furthermore, neutropenia has been recognized as a predisposing condition for infections of central venous lines,[28] and the relative risk of complication is increased for immunosuppressed patients.[36]

Several complications, both infectious and noninfectious, associated with long-term use of VAP in swine have been reported.[9,12,13,20,34] Previous attempts to use VAP in a swine model of radiation injury have been only partially successful,[9] due to thrombotic and nonthrombotic complications. Chronic antibiotics have been shown to reduce incidence of infection.[13] However, chronic antibiotic therapy is not advised in a radiation survival study, where clinical support is withheld to mimic a likely mass-casualty radiation scenario.[3,5,15] We report here surgical and postoperative procedures for VAP implantation that allow blood sampling for at least 3 mo after surgery in a Gottingen minipig model of ARS with a success rate of 95% (19 of 20 animals). To determine consequences of VAP implantation and reliability of usage in irradiated Gottingen minipigs, we monitored animals for surgical complications, sampling-related stress as assessed by monitoring of behavior, and sepsis. After euthanasia, a full necropsy was performed on all minipigs, and histopathologic examination conducted on all collected tissues to evaluate animals for any pathologic changes associated with or peripheral to VAP implantation.

Materials and Methods

Animal model. Male Gottingen minipigs ($n = 20$; age, 4 to 5 mo; weight, 9 to 12 kg) were obtained from Marshall Bioresources (North Rose, NY). Procedures were performed in accordance with protocols approved by the Armed Forces Radiobiology Research Institute (AFRRI) Institutional Animal Care and Use Committee; this institution is fully AAALAC-accredited. Minipigs were fed twice daily (Harlan Teklad Minipig diet 8753, Madison, WI) according to individual weights and provider recommendations. Minipigs were singly housed in adjoining cages that allowed tactile, visual, and auditory contact through cage bars. In accordance with our animal facility's standard operating procedures, animal enclosures were sanitized daily (during morning rounds) by using Sani-plex 128 (Quip Labs, Wilmington, DE) in Gilmour sprayers (Gilmour Group, Peoria, IL) attached to a high-pressure water system hose and appropriate scrub brushes; cages were hosed down with water each afternoon to remove all remaining excrement and food particles. Room temperature was kept at 64 to 79 °F (17.8 to 26.1 °C) and humidity at 30% to 70%.[16] Environmental enrichment and stimulation were provided in the form of physical devices (treats, sanitized toys) and positive social interaction with humans. Beginning 3 d after arrival, minipigs were acclimated to a Panepinto sling to facilitate acquisition of blood samples. The acclimation regimen consisted of placing the animals in the sling once daily for a few minutes to start and then gradually increasing the amount of time in the sling to reach 15 min per session.

The VAP (Ti SoloPort MID, Instech Solomon, Plymouth Meeting, PA) were implanted subcutaneously into the right external jugular vein 3 wk prior to irradiation. Ports were implanted just in front of the shoulder, in a position inaccessible to the animal to preclude self-trauma. Surgery was performed under general anesthesia, by using the principles of aseptic technique. Minipigs were anesthetized with tiletamine–zolazepam (6 mg/kg IM; Telazol, Fort Dodge Animal Health, Fort Dodge, IA). An intravenous catheter was placed in an ear vein, and the minipigs then were intubated. In addition, atropine (0.05 mg/kg IM; American Regent, Shirley, NY) was administered to reduce salivation. Once intubated, all minipigs were maintained on inhalant anesthesia by using 2% to 3% isoflurane (Hospira, Lake Forest, IL) and mechanical ventilation.

VAP consisted of a titanium port with a silicone septum and an attachable rounded tip silicone catheter (5 or 7 French). Both the port and catheter were flushed with 0.9% saline prior to insertion. A 3- to 4-cm incision in the right jugular groove was made at the midcervical region. Once the right external jugular vein was exposed and isolated, a ligature was placed just cranial to the proposed catheter insertion site by using 3-0 polydioxanone suture (PDS II, Ethicon, Somerville, NJ). A 2- to 3-mm incision then was made into the right external jugular vein. The catheter tip was introduced into the vein and advanced 9 to 10 cm distally. To secure the catheter within the vein, 2 ligatures of 3-0 polydioxanone were placed immediately caudal to the insertion site, on either side of the retention beads. For placement of the port, a 5- to 6-cm curvilinear incision was made just dorsal and cranial to the right scapula. Subcutaneous tissues were undermined to create a pocket for the port. The catheter was tunneled dorsally between the skin and subcutaneous tissues and attached to the port. The port then was secured to the underlying musculature by using 3-0 polydioxanone at 2 anchor points on the port. Catheter patency was confirmed intraoperatively through withdrawal of a blood sample. The port and catheter were flushed with 5 to 6 mL 0.9% saline and locked with 3 mL heparinized saline (100 U/mL, Hospira). Antibiotics were not used in the locking solution to avoid selection of resistant bacteria and to minimize the use of antibiotics in our radiation survival model, to mimic conditions expected during a radiologic or nuclear disaster. All subcutaneous tissues were closed with 3-0 polydioxanone in a simple continuous pattern. Skin incisions were closed with 2-0 polydioxanone in a continuous intradermal pattern. Tissue glue (VetBond, 3M, St Paul, MN) was used to appose any gaps in the skin incision that remained after suturing. Minipigs received a single dose of ampicillin (6 mg/kg IV; Abraxis Pharmaceuticals, Schaumberg, IL) intraoperatively to decrease the chance of surgical site infection. Once extubated, all minipigs also received a single dose of buprenorphine (0.01 mg/kg IM; Hospira) to provide analgesia. Postoperative analgesia was continued through the administration of carprofen (2 mg/kg PO; Rimadyl, Pfizer, Exton, PA) twice daily for 3 d. For prophylactic treatment in connection with VAP implantation, minipigs were given sulfamethoxazole–trimethoprim (60 mg PO; Amneal Pharmaceuticals, Hauppauge, NY) once daily for 5 d. At the time of surgery, a temperature microtransponder (Implantable Electronic ID Transponder IPPT-300, Bio Medic Data Systems, Seaford, DE) was implanted subcutaneously behind the neck, over the shoulder opposite to the site of VAP surgery, to facilitate body temperature measurements. After surgery, the minipigs were returned to their cages; no protective equipment or jackets were needed.

Irradiation and blood sampling techniques. Twenty minipigs were used for this study. For reduction purposes, 2 of the 20 animals were sham-irradiated and monitored for 30 d to serve as controls before being irradiated with ^{60}Co. Prior to irradiation, animals were anesthetized with tiletamine–zolazepam (6 to 8 mg/kg IM) and given a bolus injection of atropine (0.05 mg/kg IM) to decrease salivary secretions. All animals were given bilateral whole-body irradiation (^{60}Co, 0.6 Gy/min, 1.6 to 2.0 Gy) in our institution's ^{60}Co high dose-rate radiation

facility. Dosimetry was based on the alanine–EPR system; it was directly traceable to National Institute of Standards and Technology (United States) and National Physical Laboratory (United Kingdom) measurements. Irradiation took place 3 wk after VAP implantation. The first blood sample was obtained through the cranial vena cava 6 d prior to VAP implantation. All subsequent blood samples were collected by using VAP. The day of irradiation was considered day 0. Additional blood samples were collected on days −14, −7, and −1; at 3, 7, 11, 27, 31, and 35 h after irradiation; and on days 2, 3, 7, 10, 14, 17, 20, 24, 27, 30, 45, and 60, depending on survival. VAP were flushed at least once weekly from the time of implant, by using sterile single-use syringes and 22-gauge noncoring Huber needles with antireflux technology that were prefilled with 0.9% saline solution (10 mL Single Use, 0.9% Sodium Chloride Injection USP ZR Flush, Excelsior Medical, Neptune, NJ). Saline solution was injected with a flushing technique, where we pulse-injected about 300 to 500 µL at a time, interrupted by very brief pauses; this technique is known to increase fluid turbulence and removal of residues. The ports then were flushed with Heparin Lock Flushing Solution (100 USP U/mL, Hospira). Depending on sampling frequency, VAP were locked with either 1 or 3 mL heparin solution. Before accessing the VAP site, 5% lidocaine ointment USP (Fougera, Melville, NY) was applied topically 30 min prior to sampling, to minimize animal discomfort. Health of animals was monitored by using twice-daily visual observations (food and water intake, activity, responsiveness to human contact), once-daily clinical measurements (temperature, heart rate, respiratory rate), and monitoring of CBC and differential blood cell counts as described earlier.

Hematology, microbiology, and tissue histology. Blood was collected with strictly aseptic technique in sample tubes containing EDTA and evaluated on an automated hematology analyzer (ADVIA 120, Bayer, Pittsburgh, PA) for CBC and differential blood cell counts. Blood microbiology (aerobic and anaerobic) was assessed at days −1, 7, 14, 23, 45, and 60 or until the day of euthanasia. Tissue microbiology was performed to examine tissue alterations and infection in the proximity of the VAP. A full necropsy was performed in all cases, and histopathologic examination (hematoxylin and eosin, Masson trichrome) of all tissues was conducted in 15 of the 20 animals.

Euthanasia criteria and study endpoints. Animals were anesthetized with tiletamine–zolazepam (6 to 8 mg/kg IM) and euthanized with intravenous administration of sodium pentobarbital (Euthasol, Virbac Corporation, Fort Worth, TX) according to current American Veterinary Medical Association (AVMA) guidelines.[1] Minipigs were euthanized when any of the following criteria was present: anorexia over a 3-d period in association with lethargy, petechiation, severe thrombocytopenia, or febrile neutropenia followed by hypothermia; inability or extreme reluctance to stand persisting for 24 h (if the animal had recovered from anesthesia); core body temperature below 35.9 °C (96.6 °F) after a period of febrile neutropenia; severe acute anemia (less than 40 g/L hemoglobin, less than 13% hematocrit); presence of infection documented by blood culture and accompanied by severe systemic signs of illness; or other signs of severe organ system dysfunction with a poor prognosis.

Results

Surgery and postoperative care. To characterize the Gottingen minipig as a large animal model for radiation injury, focusing on survival at doses corresponding to the hematopoietic syndrome, the current pilot study consisted of 20 minipigs irradiated with single doses of γ rays (^{60}Co). Of these 20 minipigs, 2 were used as controls (sham-irradiated) and monitored for 30 d before being irradiated. We irradiated one pair of minipigs each month and determined their survival and circulating blood elements before irradiating the next pair. To facilitate blood sampling, we surgically implanted subcutaneous VAP in 20 Gottingen minipigs and waited for the wound to heal before exposing them to radiation. Because of the life-extending effect of antibiotics in radiation victims and their presumed unavailability in a mass casualty scenario, we evaluated whether it was possible to completely omit antibiotic-based prophylactic care after surgery for VAP implantation or whether a short-term treatment followed by a long recovery period without drugs was essential. The first pair of minipigs did not receive prophylactic treatment; of these 2 animals, one remained healthy until the scheduled irradiation procedure, whereas the other exhibited signs of sepsis (*Staphylococcus aureus*) 2 d after surgery, thereby indicating the need for postoperative antimicrobial care for the remaining animals. The remaining 18 animals in the study received a postoperative prophylactic regimen of oral carprofen (22.5 mg twice daily for 3 d) and oral sulfamethoxazole–trimethoprim (60 mg once daily for 5 d) and did not show any signs of infection prior to irradiation. Surgical wounds were monitored daily for signs of edema and purulent material. There were no indications of infection for these 18 animals, as was confirmed by negative blood culture results. Irradiation followed 3 wk after surgery, to allow time for the wound to heal and the animals' metabolisms to clear residual traces of drugs.[23,37] Comparison of hematologic values obtained before and 2 wk after surgery showed no significant changes in hematologic parameters (Table 1) by Student *t* test ($P > 0.05$ for all tested parameters). Values were within reported ranges for healthy Gottingen minipigs.[10]

VAP selection, functionality, and blood collection. The model, dimensions, and proper maintenance of the device are critical for the success of blood collection and are subject to species-specific variations, as they affect accessibility of the port, impact the tissue at the site of implantation, and influence the likelihood of clot formation.[29] We selected VAP dimensions and capacity (Figure 1) according to the animal weight at time of surgery and throughout the duration of the study (9 to 15 kg). The soft silicone catheter with round tip was chosen to reduce irritation of the tissue at the site of insertion, and inflammatory complications; precoating the catheter with heparin to reduce clotting was not necessary.

At the time of sampling or flushing, minipigs were restrained in a Panepinto sling. A majority of VAP-related infections have been linked to *Staphylococcus* microorganisms;[26] therefore, we used topical application of povidone–iodine as antibacterial treatment to clean the skin at the site of the subcutaneous port, according to a previously published procedure.[21] The site was scrubbed 3 times with povidone–iodine; after the last scrub, povidone–iodine was allowed to dry for 5 to 7 min; the area then was wiped with 70% ethanol until all traces of scrub solution were removed.

When blood collection was required, the first 1 to 2 mL blood from the VAP was discarded, and an additional 3 to 8 mL (depending on the frequency of sampling) was drawn. Current recommendations for blood sampling are to not to exceed 1% of total blood volume (calculated as 65 mL/kg for swine) when doing multiple samplings daily and to limit overall blood withdrawal to 7.5% total body volume per week.[19] With day 0 being the day of irradiation, we collected 9 consecutive 4-mL samples in 5 d by bleeding minipigs (9 to 15 kg) once before irradiation, 3 times on day 0, 3 times on day 1, and once daily on days 2 and 3, for an overall volume (36 mL) corresponding

Table 1. Blood cell counts ($\times 10^3$ cells/μL; mean ± 1 SD) in minipigs before and 2 wk after VAP surgery

	Before surgery	After surgery	Normal range[a]
WBC	10.29 ± 1.88	9.88 ± 1.52	7.2–16.5
Lymphocytes	5.16 ± 0.73	5.20 ± 0.91	5.3–12.3
Neutrophils	4.49 ± 1.49	3.68 ± 1.23	1.3–6.2
Eosinophils	0.14 ± 0.06	0.21 ± 0.11	0–0.4
Monocytes	0.33 ± 0.12	0.32 ± 0.09	0–4
Platelets	579.28 ± 112.06	553.56 ± 105.33	413–684

[a]Normal values obtained from reference 10.

Body	Material: titanium Height: 0.395 in. (10 mm) Diameter: 2.5 cm Dead volume: 0.38 mL Weight: 6.7 g
Catheter	Type: attachable, round tip Sizes: 5 and 7 French Material: silicone Volume: 0.7 mL
Septum	Material: silicone Diameter: 0.36 in.

Figure 1. Specifications of VAP model used in the current study.

to less than 6% total volume during 1 wk. This number of time points for hematology was necessary to obtain adequate data to establish the minipig as a standard radiation injury model, in view of the importance of lymphocyte depletion dynamics and the different half-lives and time courses of circulating levels of various blood elements.[4] During the remainder of the study, blood samples were obtained at different time intervals, ranging from twice a week to once every 2 wk. Depending upon sampling frequency, VAP were locked with either 1 or 3 mL heparin solution to maintain consistency in the total volume of heparin injected within a 24-h period throughout the study. Before and after blood collection, the device was flushed with 8 mL saline solution by using single-use syringes with antireflux technology to eliminate any chance of cross-contamination.

Blood draws were performed throughout the duration of the study, which varied between approximately 1 to 3 mo, depending on animal survival after irradiation, with the total number of bleeds per animal varying between 12 and 20. VAP were fully functional in 19 of the 20 cases and partially functional (9 successful samplings, during the first 3 wk after surgery) in the remaining minipig; progressive occlusion of the catheter led to eventual failure 3 d after irradiation (Table 2). Histopathologic examination of the point of occlusion found chronic thrombosis of the vessel with proliferative vasculopathy. Microscopic analysis of the catheter wall indicated the presence of a pinpoint-size hole, which led to blood leakage and clot formation.

Stress response to blood sampling. Swine are very sensitive to stressors such as shipping, handling, pain, surgery, and trauma. In healthy pigs, the concentration of lymphocytes is higher than that of neutrophils; venipuncture may result in a mild stress response and a doubling of the circulating neutrophil count within 30 min, lasting for 8 h.[11]

In stressed animals, leukograms are characterized by coexisting mature neutrophilia, lymphopenia, and eosinopenia, resulting in a decreased lymphocyte:neutrophil ratio.[18] We calculated the lymphocyte:neutrophil ratio from 20 animals

Table 2. Blood collection and sampling efficiency

Minipig	^{60}Co dose (Gy)	Duration of VAP usage (d)	No. of samples collected/no. expected
1	1.6	78	19/19
2	1.6	78	19/19
3	1.6	79	19/19
4	1.6	79	19/19
5[a]	1.7	93	33/33
6	1.7	51	9/18
7	1.7	51	18/18
8	1.8	57	18/18
9	1.8	50	16/16
10	1.8	37	11/11
11	1.8	38	15/15
12	1.8	54	16/16
13	1.8	65	19/19
14[a]	1.9	90	34/34
15	2.0	35	15/15
16	2.0	30	13/13
17	2.0	40	15/15
18	2.0	40	15/15
19	2.0	41	16/16
20	2.0	35	14/14

The rate of successful sampling was 100% in 19 of 20 minipigs; the rate was 50% in minipig 6 due to leakage, clotting, and occlusion of the catheter of the VAP. The only other VAP-associated complications were cases of sepsis in minipigs 16 and 18.
[a]These minipigs were used as sham-controls and were followed for 30 d before being irradiated with ^{60}Co.

bled once weekly for 2 wk prior to irradiation (ratio, 1.5 ± 0.7) and from 2 sham-irradiated minipigs bled 9 times in 5 d (Table 3). When the 2 sham-irradiated minipigs were sampled 3 times daily, the number of circulating neutrophils increased for both animals after the second or third blood sample and returned to levels within normal ranges overnight. One animal showed reduction in the lymphocyte:neutrophil ratio, but neutrophilia was minimal and not concomitant with lymphopenia and eosinopenia. In addition temperature, heart rate, and respiratory rate were measured at each blood draw (Table 4). Variations between single and multiple bleedings daily were consistent with very mild handling-associated stress in all 20 animals.

Histopathologic evaluation of tissue surrounding VAP and microbiologic analysis of blood and tissues. Appropriate choice of port-dome height and correct placement of sutures, reduction of the dead space, and immobilization of the VAP are essential to reduce local inflammation, seroma formation, tissue tension, and necrosis.[29] To evaluate the quality of implantation in our model, we performed histopathology and microbiology of tissues and fluids at the site of VAP implantation. In addition, at various times during the survival study (days −1 to 60), we tested blood for presence of infectious organisms. In 19 of the 20 cases, the VAP and surrounding tissue appeared healthy, with focal granulation tissue, minimal cellular inflammation, and mild subacute hemorrhages. On histologic examination of the skin and subcutis surrounding the VAP in these cases (Figure 2), the metal implant consistently was surrounded by a dense rim of granulation tissue and fibrosis. Histopathologic analysis showed normal wound healing and fibrosis surrounding the port.

Table 3. Circulating blood elements in sham-irradiated minipigs

	Day of sampling	No. of hours after first sampling	No. of cells ($\times 10^3/\mu L$)			Lymphocyte:eosinophil ratio
			Lymphocytes	Neutrophils	Eosinophils	
Minipig 1	Baseline[a]	not applicable	3.8–5.9	2.7–5.7	0.07–0.18	not calculated
	1	0	5.45	4.29	0.18	1.27
	2	27	4.99	5.78	0.17	0.86
		31	4.9	5.77	0.19	0.85
		35	5.42	9.6	0.18	0.56
	3	51	5.62	3.19	0.35	1.76
		55	6.1	6.92	0.15	0.88
		59	5.77	6.54	0.16	0.88
	4	72	4.73	3.92	0.21	1.21
	5	96	4.36	3.66	0.25	1.19
Minipig 2	Baseline[a]	not applicable	4.2–5.7	1.7–4.0	0.11–0.31	not calculated
	1	0	4.64	3.59	1.6	1.29
	2	27	3.34	1.83	1.9	1.83
		31	5.34	2.09	0.6	2.56
		35	6.28	2.65	0.6	2.37
	3	51	5.87	1.35	2.2	4.35
		55	6.5	1.82	0.9	3.57
		59	5.91	2.28	0.2	2.59
		72	5.72	1.71	2.1	3.35
		96	5.7	1.83	1.6	3.11

[a]Baseline values were established by using 4 single time points taken at 1-wk intervals before irradiation.

Two pigs developed VAP-related systemic infection attributed to *Staphylococcus aureus*; one minipig became septic after irradiation; the other, which was 1 of the 2 animals for which antibiotic treatment was waived, was already septic at the time of irradiation. Of the 2 animals with histopathologic evidence of sepsis, 1 had a suppurative vasculitis in the catheterized vessel; both animals were diagnosed as having vegetative valvular endocarditis with intralesional gram-positive cocci. There was evidence of embolic showering of bacteria in both animals, resulting in a suppurative pneumonia in one case and a necrotizing orchitis in the other. The remaining minipigs showed no evidence of VAP-associated systemic pathology.

Microbiologic analysis of blood drawn at various times prior to the end of the study revealed the presence in 7 cases of trace numbers of coagulase-negative *Staphylococcus*; however, the presence of bacteria was sporadic, and the events were few and isolated. Affected minipigs were afebrile and did not exhibit any other clinical signs. Full-body necropsy and histopathologic evaluation of all tissues ruled out the possibility of infection.

Discussion

We assessed feasibility of repeated blood sampling in irradiated animals over a period that varied from 5 wk to 3 mo. We expected that the extensive immunosuppression, thrombocytopenia, and acute inflammation that characterize victims of radiation injury would exacerbate infectious and thrombotic complications associated with long-term use of VAP.[9] However, using strictly aseptic technique when accessing VAP, flushing and locking VAP at least once each week, and using single-use syringes containing sterile saline solution allowed us to obtain multiple blood samples in 19 of 20 severely immunocompromised and thrombocytopenic minipigs (lymphocytes less than 1.5×10^3 cells/μL, neutrophils less than 0.6×10^3 cells/μL, platelets less than 4×10^3 cells/μL), reaching an efficiency of 95%.

Choice of proper VAP design and extreme care in minimizing the amount of subcuticular dead space around the device were essential to limit complications. Histologic findings confirmed the lack of tissue necrosis and infection surrounding the device and normal healing of the wound. The area of septum and angle of access proved key in terms of length of sampling time and anesthesia requirements. We evaluated several designs before selecting the device used for this study. In initial tests, we used

Table 4. Vital signs at blood sampling of sham-irradiated minipigs (n = 2)

	Single daily sample			Multiple daily samples		
	Median	Mean	1 SD	Median	Mean	1 SD
Heart rate (bpm)	122	121.7	6.7	90	96	16.4
Respiration rate (breaths per minute)	34	32.3	8	27	27.6	6
Temperature (°C)	38.1	38.1	0.4	38.1	38.1	0.5

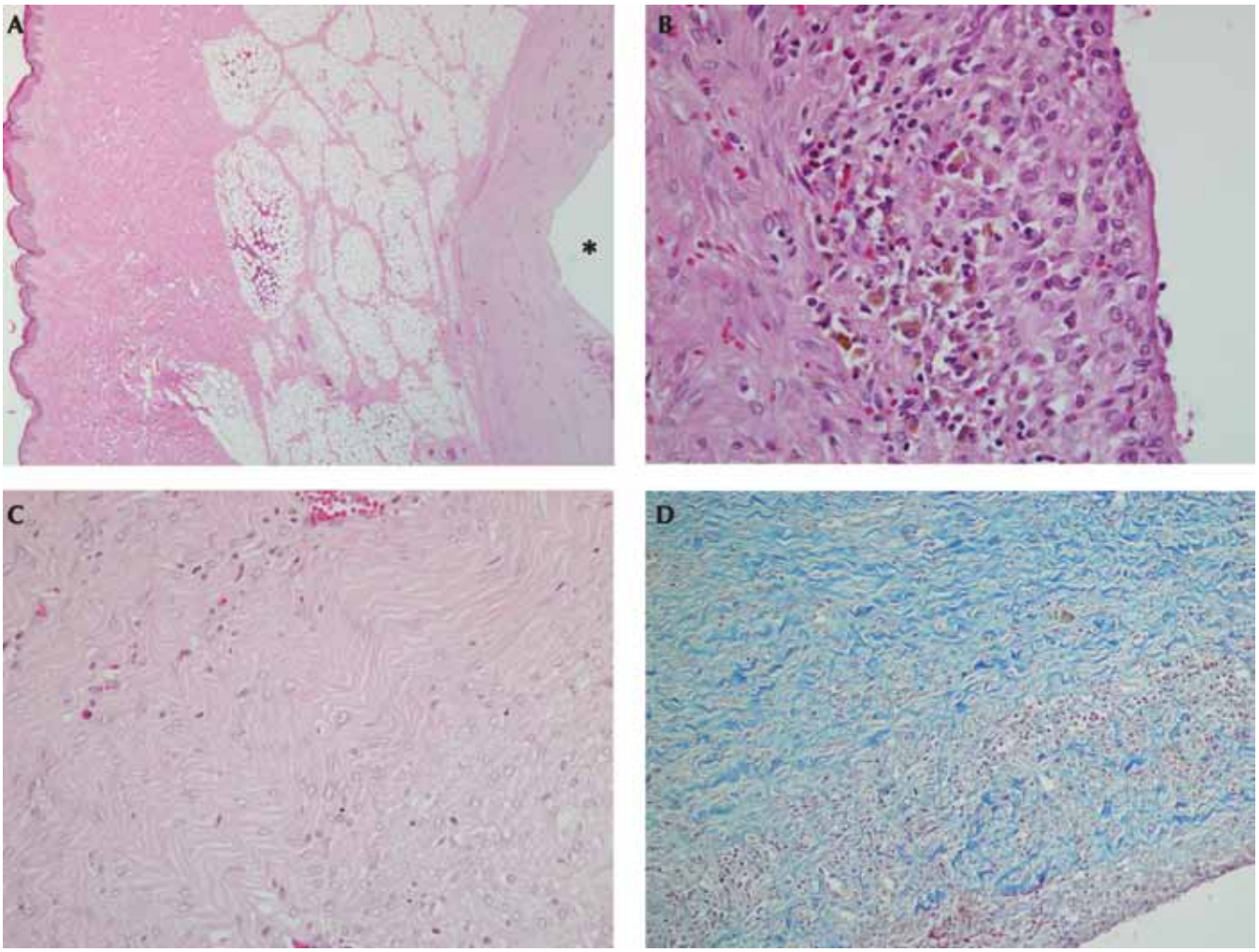

Figure 2. Histologic examination of the skin and subcutis surrounding VAP in minipigs. (A) Haired skin and subcutis. The position of the VAP (*) is bounded by an immediate layer of granulation tissue and further surrounded by dense strata of fibrous connective tissue and fibrosis. Hematoxylin and eosin stain; magnification, ×20. (B) Layer of granulation tissue composed of reactive fibroblasts, histiocytes, hemosiderophages, lymphocytes admixed with loose collagen, and small caliber vessels (arrows) immediately adjacent to the VAP. Hematoxylin and eosin stain; magnification, ×400. (C) Organized, undulating strata of fibrous connective tissue with regularly spaced small caliber capillaries and fibroblasts. Hematoxylin and eosin stain; magnification, ×400. (D) Gradations of fibroplasias (blue), which increase in density as distance from the VAP increases, compared with the paler blue of the loose collagen admixed with increased density of small and loose capillaries near the surface of the wound. Masson trichrome stain; magnification, ×200.

a VAP model that provided decreased turbulence in the port, reducing incidence of clotting; however, the septum was small and positioned almost perpendicular to the skin, making it difficult to obtain blood and necessitating the use of anesthesia for each blood collection. Subsequently, we used a VAP design with a larger septum oriented parallel to the surface; sampling from this device did not require anesthesia of the minipigs.

Prophylactic use of antibiotics and antiinflammatories in connection with VAP implantation was necessary to reduce swelling, wound tension, and tissue necrosis and ultimately to ensure sterility. As a part of the study to establish the minipig as a valuable model to study radiation injury, we determined the requirement for prophylactic use of antibiotics and nonsteroidal antiinflammatory drugs in conjunction with VAP surgery. Antibiotics are known to improve survival among victims of radiation injury; for this reason we considered postsurgery prophylactic treatment as a possible confounding factor. The treatment was waived for 2 minipigs; shortly after surgery, 1 of the animals became heavily infected with *Staphylococcus aureus*, indicating that prophylactic care should not be avoided. The recovery time between surgery and irradiation was set to 3 wk, to allow for complete elimination of drugs from the body systems[23,37] and healing of the surgical wound. In several cases, transient trace amounts of *Staphylococcus* spp. were cultured at

various points during the course of the experiment; however, the majority of these cases was negative on the terminal blood draw and had no histopathologic findings to support sepsis. As such, we believe that the blood samples with transient positive cultures for *Staphylococcus* spp. were spurious, associated with environmental contamination from the skin of the animal[22] or the individual drawing the sample.

We modified and enhanced reported procedures for VAP implantation and use in irradiated and immunocompromised minipigs.[9] The main changes consisted of replacing the material used for suturing the catheter (polydioxanone instead of silk), eliminating butterfly needle-mediated chronic port access, using single-use sterile prefilled syringes with antireflux technology for flushing devices, performing strictly aseptic weekly maintenance of the device, and acclimating minipigs to the sling to reduce stress and facilitate sampling. The large volume of flushing solution and pulse-flushing technique used may have reduced chances of thrombus formation and infectious complications, given that the relationship between the 2 conditions is well established.[25] The incidence of infectious and noninfectious complications was reduced to 10% (2 of 20 minipigs), as compared with that of previous studies (56%, 5 of 9 animals). Sampling required less than 15 min per minipig, including measurement of vital signs, and did not require anesthesia. Stress to the animals was minimal, as supported by lack of coexisting mature neutrophilia, lymphopenia, and eosinopenia.[18]

The hematopoietic acute radiation syndrome consists of a combination of acute immunodeficiency, tissue injury, and coagulopathies. Depending on the severity of the damage, radiation victims may succumb to infection, massive hemorrhage, and organ failure.[4] A wider choice of long-lived large animal models is expected to expedite advanced drug development of pharmacologic radiation countermeasures; swine have been suggested as one of the most promising species for drug testing.[2] Current medical management of radiation victims is based primarily on clinical signs and hematologic evaluation.[35] Therefore, assessment of CBC and differential blood cell counts is essential to establish minipigs as a model system. The uniqueness of ARS, in conjunction with the depth of vessels suitable for repeat phlebotomy (that is, vena cava or jugular veins) in swine, poses a risk of persistent hemorrhage and ultimately may lead to hypovolemia in cases when multiple samples must be obtained over a short period of time. Establishment of a procedure for obtaining blood samples at the required frequency and without affecting survival is therefore necessary. Previous attempts to use Yucatan minipigs to study ARS were only partially successful, because of the difficulties encountered in obtaining a sufficient number of samples and maintaining functionality of VAP.[9] Nevertheless, that experience was valuable in establishing guidelines for a technique for VAP implantation, maintenance, postoperative care, and blood sampling in irradiated Gottingen minipigs.

We conclude acute immunosuppression and coagulopathies do not preclude the use of VAP in minipigs and that serial blood sampling is feasible in a minipig model of ARS.

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