Journal of the American Association for Laboratory Animal Science
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Vol 52, No 1
January 2013
Pages 63–69

Clean Technique for Prolonged Nonsurvival Cardiothoracic Surgery in Swine (*Sus scrofa*)

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Laboratory animal regulations provide little guidance regarding duration of nonsurvival surgery requiring aseptic technique. We hypothesized that swine would experience no sepsis during nonsurvival cardiothoracic surgery accomplished by using clean technique and lasting 8 h or less. Incision sites of 5 male farm pigs (*Sus scrofa*) were shaved and then cleaned with alcohol and povidone–iodine. The surgeon wore sterile gloves, clean scrubs, and hair bonnet; assistants wore clean scrubs and nonsterile gloves; most instruments were autoclaved. A median sternotomy incision was used for thoracic cavity exposure, and the skull was exposed to allow induction of brain death. Heart rate, body temperature, and blood samples were obtained before surgery (0 h; baseline) and at 2, 4, 5 or 6, and 7 or 8 h thereafter. Statistical analysis by *t* tests showed that heart rate was unchanged and body temperature increased after the 0-h (baseline) time point. Aerobic blood cultures were negative except for 2 samples that were positive for coagulase-negative *Staphylococcus* spp. at 4 h. RBC, Hgb, and Hct levels were decreased at 2 and 4 h, but WBC and platelets were unchanged. Other alterations included decreased glucose (at 7 or 8 h), increased BUN (at 5 or 6 h and 7 or 8 h) and creatinine (at 5 or 6 h), decreased Na^+ and Ca and increased K^+ (most time points), decreased total protein and albumin (most time points), and decreased globulin (at 7 or 8 h). Liver enzymes and bilirubin typically were unchanged, and cholesterol consistently was decreased. Together our results indicate a lack of sepsis for 8 h or less in pigs undergoing cardiothoracic surgery by using clean technique. These findings provide new and specific data regarding the use of aseptic technique during prolonged nonsurvival surgeries.

Any successful surgical procedure must accomplish the goals of the operation while minimizing surgical complications, one of the most serious of which is sepsis. Sepsis has been defined as "the systemic inflammatory response syndrome [that] is the result of a confirmed infectious process."[5] Sepsis has multiple causes, but one of the most common is bacteremia, the presence of viable bacteria in the blood. Because bacteria gain access to inner tissues and blood with any disruption of dermal integrity, such as occurs during surgery, measures must be taken to prevent perioperative bacterial contamination. These measures collectively are known as aseptic technique: the methods and practices that prevent cross-contamination during surgery.[12]

Although aseptic technique is the standard of care for survival surgeries in animals, laboratory animal science regulations and literature provide little guidance regarding the type or duration of nonsurvival surgery that requires the use of aseptic surgical technique. The *Guide for the Care and Use of Laboratory Animals*[16] acknowledges that all of the procedures listed for aseptic technique during survival surgeries may not be necessary for nonsurvival procedures; however, "at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean." Otherwise, the language is vague: "For nonsurvival procedures of extended duration, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome."[16] The American College of Laboratory Animal Medicine Position Statement on Rodent Surgery[2] simply states that all rodent survival surgery should incorporate aseptic technique and makes recommendations for how the attending veterinarian or designee, laboratory animal medicine specialists, and the IACUC should contribute to the formulation of institutional rodent surgical standards and standards for the qualification and training of personnel involved in survival surgery or perioperative care. However, this statement does not address nonsurvival surgery or surgeries in nonrodent species.

Regarding survival surgery, the *Guide for the Care and Use of Laboratory Animals*[16] states that inadequate or incorrect aseptic technique may lead to subclinical infections causing physiologic and behavioral changes that can influence "surgical success, animal well-being, and research results." Therefore, aseptic technique is required for survival surgery in any laboratory animal species, to protect against postoperative infection. The Animal Welfare Act and Regulations[3,4] simply state that personnel at research institutions involved in animal care and use must receive adequate training for their duties, including training in aseptic surgical methods and procedures and instruction on "research or testing methods that minimize or … limit animal pain or distress."

At our institution, rodent survival surgery requires sterilized instruments; a clean, uncluttered surgical space dedicated to surgery for the duration of the surgical procedure(s); and a separate preparation area. The surgical site is prepared by removing hair and cleaning with alternating alcohol and antiseptic solution. The surgeon must wear a clean lab coat, gown, or scrubs; a face mask; and sterile surgical gloves during surgery. Survival surgery on USDA-regulated species requires sterilized instruments and a dedicated surgical suite that includes the operating room and separate areas for animal preparation, surgeon preparation, and animal recovery. The surgical site is prepared by removing hair and cleaning with alternating alcohol and antiseptic solution. The surgeon must wear facility scrubs, a sterile surgical gown, a face mask, shoe covers, and a hair

Received: 28 Mar 2012. Revision requested: 09 May 2012. Accepted: 05 Jul 2012.
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bonnet during surgery. In addition, the surgeon must perform a thorough hand scrub prior to donning sterile surgical gloves for rodent survival surgery.

For nonsurvival surgery on any species at our institution, surgeon attire and patient preparation must be described in the protocol, but strict aseptic technique typically is not required, and some procedures may instead be conducted in a 'clean' manner. However, clear guidance is not available for nonsurvival surgeries of extended duration, and standards for these surgeries are determined on a case-by-case basis. With the current project, we sought to address the use of clean technique for nonsurvival surgery.

We hypothesized that swine that underwent nonsurvival cardiothoracic surgery by using clean technique would show no evidence of bacteremia or sepsis, even after an extended duration (8 h). The goal of the current study was to test this hypothesis by evaluating serial blood cultures and patient clinical parameters, including hematologic and clinical chemistry profiles, for evidence of bacteremia or sepsis at regular time points after the initiation of nonsurvival cardiac surgery in swine. These results are intended to provide new and more specific information relevant to the acceptability of clean technique during prolonged nonsurvival surgeries.

Materials and Methods

Animals. Intact male Yorkshire–Landrace crossbred farm pigs (*Sus scrofa*; *n* = 5; weight, 35.0 to 56.5 kg) were obtained from a closed herd (Washington State University, Pullman, WA). The pigs were conventionally housed at the vendor and vaccinated for *Bordetella bronchiseptica*, *Pasteurella multocida*, and *Erysipelothrix rhusiopathiae* (Rhinogen BPE, Merck Animal Health, Summit, NJ). The pigs were subjects of an approved nonsurvival study evaluating systemic cytokine release and changes in cardiovascular and organ function parameters resulting from brain death, during which serial blood samples were collected throughout the experiment. All procedures in this project were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*[16] and approved by the IACUC of the University of Washington, an AAALAC-accredited institution.

Preoperative procedure. After arrival at our institution, pigs were allowed at least 5 d to acclimate prior to surgery. Food was withheld from all pigs the evening before surgery, and water was withheld the morning of surgery. Anesthesia was induced with tiletamine and zolazepam (5.5 mg/kg IM; Telazol, Fort Dodge Animal Health, Overland Park, KS) and xylazine (5.5 mg/kg IM; AnaSed, Lloyd Laboratories, Shenandoah, IA). No antimuscarinic agents (atropine, glycopyrrolate) were used. All pigs were intubated, anesthetized with isoflurane (1.5% to 2% in 100% oxygen), and maintained on a ventilator throughout the procedure. Intravenous catheters were placed in marginal ear veins for perioperative delivery of warmed lactated Ringer solution (10 mL/kg hourly). Heat support was provided by using a Bair Hugger (Arizant Healthcare, Eden Prairie, MN) maintained on the 'high' setting (109.4 ± 5.4 °F). Pigs were restrained in dorsal recumbency, and surgical sites were clipped and cleaned with alternating alcohol and 7.5% povidone–iodine surgical scrub (Vetadine, Vedco, St Joseph, MO). Surgeon attire consisted of sterile gloves, clean scrubs, and a hair bonnet. Surgical assistants wore clean scrubs and nonsterile laboratory gloves. Most instruments were autoclaved prior to surgery and arranged on a sterile field, except for large bandage scissors used during sternotomy, a sternal retractor, and a craniotomy burr; these items were clean but not sterile.

Surgical procedure and monitoring. Continuous animal monitoring included electroencephalography, electrocardiography and heart rate, indirect blood pressure, end-tidal CO_2, oxygen saturation, and rectal temperature (SurgiVet Advisor Vital Signs Monitor, Smiths Medical, Dublin, OH).

A midline ventral thoracic skin incision was made from manubrium to xiphoid, and a midline sternotomy was performed to expose the thoracic cavity for direct manipulation and instrumentation of the heart. A longitudinal pericardiectomy was performed, and the pericardium was suspended. A left ventricular pressure–volume catheter (ADI Instruments, Colorado Springs, CO) was inserted into the left ventricular apex through a pursestring suture. Approximately 60 to 90 min after the initial skin incision, brain death was induced. Briefly, a midline skin incision was made on the cranium and the temporal musculature bluntly dissected until the calvarium was visualized. A craniotomy was made in the frontoparietal region by using a 4-mm burr, and an 8-French Foley balloon catheter was inserted into the subdural space. Brain death was achieved by gradually inflating the balloon with 20 mL sterile saline over a 20-min period and confirmed by relative flattening of the electroencephalogram and the development of fixed mydriasis. Isoflurane anesthesia, physiologic monitoring, and blood sampling continued at scheduled time points for the duration of the experiment (maximum, 8 h). At the end of surgery, anesthetized pigs were euthanized by injection of high-potassium (University of Wisconsin cardioplegia) solution[18] into the aortic root, with a distal aortic cross-clamp in place.

Blood samples. Blood samples (10 to 12 mL each) were obtained every 1 or 2 h from the right or left femoral artery or vein, the cranial vena cava, or the left ventricle of the heart by using sterile syringes and needles. A baseline (time 0) blood sample was obtained prior to surgical incision and after each pig was intubated, anesthetized with isoflurane, and clinically stable. The start of surgery was designated as the time the thoracic cavity was exposed, which occurred 5 to 10 min after the 0-h time point, and sampling times were calculated from that point. Perioperative blood samples were obtained at 2 and 4 h from all 5 pigs; at 5 (1 pig) or 6 (4 pigs) h; and at 7 (2 pigs) or 8 (2 pigs). One 8-h sample was drawn immediately postmortem from the left ventricle of a euthanized pig within 10 min of administering cardioplegia solution. Baseline values (*n* = 5) were compared with those obtained at 2 (*n* = 5), 4 (*n* = 5), 5 or 6 (*n* = 5), and 7 or 8 (*n* = 4) h after the start of surgery.

Heart rate and body temperature. Heart rate and body temperature typically were recorded at the time of blood sampling. Heart rate was obtained from electrocardiographic recording, and body temperature was measured by using a rectal probe. Baseline heart rate and body temperature values were compared with those obtained at 2 (*n* = 5), 4 (*n* = 5), 5 or 6 (*n* = 3), and 7 or 8 (*n* = 3) h after the start of surgery.

Blood cultures. Blood (1 to 3 mL) from each sampling time was added to a sterile blood culture vial and submitted to a commercial laboratory (Phoenix Central Laboratory, Everett, WA, or Specialty VetPath, Shoreline, WA). Blood was injected into the culture vial before other blood tubes, and the rubber stopper was swabbed with an alcohol pad prior to introduction of the blood sample. Results were considered negative when no growth occurred in 7 d (Phoenix Central Laboratory) or 5 d (Specialty VetPath). Culture results at baseline (*n* = 5) were compared with those obtained at 2 (*n* = 5), 4 (*n* = 5), 5 or 6 (*n* = 5), and 7 or 8 (*n* = 4) h after the start of surgery.

Complete blood count and WBC differential. Blood (2 to 3 mL) from each sampling time was added to an EDTA-containing tube

Table 1. Heart rate (bpm) and body temperature (°F) in swine undergoing cardiothoracic surgery.

	Time (h) after start of surgery					
	0 (n = 5)	2 (n = 5)	4 (n = 5)	5 or 6 (n = 3)	7 or 8 (n = 3)	Reference range[14,26]
Heart rate	104 ± 21.7	103.6 ± 18.4	102 ± 27.2	109.7 ± 14.5	115 ± 4.6	105 ± 10
Body temperature	98.7 ± 0.8	99.9 ± 0.6[a]	100.2 ± 1.0[a]	100.8 ± 0.3[a]	101.7 ± 1.3[a]	102.6 ± 0.9

Data are given as mean ± 1 SD.
[a]Value significantly ($P < 0.05$) different from that at 0 h.

Table 2. RBC count (x 10^6/µL), Hgb concentration (g/dL), and Hct (%) in swine undergoing cardiothoracic surgery

	Time (h) after start of surgery					
	0 (n = 5)	2 (n = 5)	4 (n = 5)	5 or 6 (n = 5)	7 or 8 (n = 4)	Reference range[1]
RBC	6.5 ± 0.5	5.9 ± 0.3[a]	5.8 ± 0.5[a]	5.9 ± 0.5	5.9 ± 1.0	6.0 ± 1.0
Hgb	11.1 ± 0.8	10.0 ± 0.6[a]	9.7 ± 0.6[a]	10.0 ± 0.9	9.9 ± 1.8	11.0 ± 2.0
Hct	34.9 ± 2.1	31.4 ± 1.5[a]	30.4 ± 2.4[a]	31.9 ± 2.4	31.6 ± 5.1	39.5 ± 3.5

Data are given as mean ± 1 SD.
[a]Value significantly ($P < 0.05$) different from that at 0 h.

and submitted to Phoenix Central Laboratory for analysis. CBC and WBC differential values at 0 h (n = 5) were compared with those obtained at 2 (n = 5), 4 (n = 5), 5 or 6 (n = 5), and 7 or 8 (n = 4) h after the start of surgery. A few individual values were unavailable due to hemolysis or platelet clumping.

Clinical chemistry profiles. Blood (2 to 3 mL) from each sampling time was added to a serum-separator tube and submitted to Phoenix Central Laboratory for analysis. Clinical chemistry values at baseline (n = 5) were compared with those at 2 (n = 5), 4 (n = 5), 5 or 6 (n = 5), and 7 or 8 (n = 4) h after the start of surgery. A few individual values were unavailable due to hemolysis.

Statistical methods. Means and standard deviations were calculated for all numerical data and compared with baseline values by using the 2-tailed t test (Excel, Microsoft, Redmond, WA). A P of less than 0.05 was defined as the level of significance.

Results

Heart rate. In humans, increased heart rate is one of several clinical manifestations that characterize sepsis.[5] We therefore recorded heart rate at regular time points during prolonged cardiothoracic surgeries in swine, both as an experimental parameter and as routine monitoring during surgery. Heart rate at 2, 4, 5 or 6, or 7 or 8 h after the start of surgery did not differ from the baseline value (Table 1). Heart rates at all time points were consistent with reference values for resting heart rate in normal pigs (Table 1).[26]

Body temperature. Because body temperature may increase or decrease with sepsis,[5] we recorded body temperature at regular time points during these prolonged surgeries in our swine. Body temperature at 2, 4, 5 or 6, and 7 or 8 h was significantly ($P < 0.05$) higher than that at baseline (Table 1). All values except those at hours 7 or 8 were lower than the normal reference range for body temperature in swine (Table 1).[14]

Blood culture. Blood cultures are the standard diagnostic method used to evaluate suspected clinical cases of bacteremia and septicemia. Blood cultures were therefore submitted at regular time points during these prolonged procedures. There was no growth of aerobic bacteria in any blood cultures sampled at 0, 2, 5 or 6, or 7 or 8 h. However, 2 of 5 blood cultures sampled at 4 h yielded growth of coagulase-negative *Staphylococcus* species; one of these positive cultures was identified as 'scant growth.'

CBC and WBC differential. The CBC is a standard diagnostic method used to evaluate changes in blood components indicative of clinical disease, including septicemia. Marked increases or decreases in total WBC count are associated with sepsis in human patients.[5] We therefore evaluated the CBC at scheduled time points during these prolonged surgeries. RBC count, Hgb, and Hct were all decreased ($P < 0.05$) at the 2- and 4-h time points compared with baseline values (Table 2). RBC and Hgb values at all time points are consistent with reference values in swine (Table 2),[1] as were Hct values at baseline and hour 7 or 8.

Compared with baseline values, there was no significant change in total WBC or platelet counts at the 2-, 4-, 5- or 6-, or 7- or 8-h time points (Table 3). All values were consistent with normal reference ranges for total WBC and platelet counts in swine (Table 3).[1]

A WBC differential analysis provides relative percentages of leukocytes, which may be altered in a variety of disease processes. For example, in humans, a relative increase in immature (band) leukocytes to more than 10%, with or without leukocytosis, is consistent with sepsis.[5] We therefore obtained a differential analysis at scheduled time points during the prolonged surgeries. Results showed no significant change in the relative percentages of neutrophils, band neutrophils, lymphocytes, eosinophils, or basophils at 2, 4, 5 or 6, or 7 or 8 h after the start of surgery, compared with baseline values. Monocytes were decreased ($P < 0.05$) only at the 7- or 8-h time point compared with the baseline value (Table 3). All WBC differential values are similar to respective reference values in swine (Table 3).[1]

Clinical chemistry profile. Severe sepsis can be associated with single- or multiple-organ dysfunction and failure;[5] therefore, clinical chemistry assays are valuable standard diagnostic tests to evaluate changes in organ function that are indicative of sepsis. We evaluated clinical chemistry values at sequential time points during these prolonged surgeries. Results showed decreased ($P < 0.05$) glucose at 7 or 8 h after the start of surgery, increased ($P < 0.05$) BUN at the 5- or 6-h and 7- or 8-h time points, and increased ($P < 0.05$) creatinine at 5 or 6 h, compared with the corresponding baseline (time 0) value (Table 4). Glucose, BUN, and creatinine values were consistent with reference values for swine at all time points; however, glucose was higher than the reference range at the 2-h time point and lower than the reference at 7 or 8 h (Table 4).[1]

Changes in electrolytes included decreased ($P < 0.05$) Na^+ at hours 2, 4, and 5 or 6; increased ($P < 0.05$) K^+ at hours 2, 4, 5 or 6, and 7 or 8; and decreased ($P < 0.05$) Ca^{2+} at hours 2, 4, 5 or 6, and 7 or 8, compared with the corresponding time 0 values

Table 3. WBC count (x 10^3/μL), platelet count (x 10^3/μL), and WBC differential values (%) in swine undergoing cardiothoracic surgery

	Time (h) after start of surgery					
	0 (n = 5)	2 (n = 5)	4 (n = 5)	5 or 6 (n = 5)	7 or 8 (n = 4)	Reference values[1]
WBC	12.1 ± 1.2	11.0 ± 2.2	12.2 ± 3.2	14.0 ± 2.2	18.2 ± 6.0	16.5 ± 5.5
Platelets	273 ± 99	263 ± 41.4[b]	243 ± 59[b]	206 ± 87.5	201 ± 71.3[c]	350 ± 150
Neutrophils	30.3 ± 15.0	34.3 ± 14.3	35.1 ± 16.5	24.1 ± 5.8	21.8 ± 9.2	45.0 ± 25.0
Band neutrophils	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 2.0
Lymphocytes	62.8 ± 15.6	60.1 ± 14.5	60.3 ± 15.4	72.2 ± 6.5	75.8 ± 9.0	55.0 ± 20.0
Monocytes	5.7 ± 2.2	4.6 ± 1.7	3.5 ± 2.1	3.8 ± 1.3[c]	1.9 ± 1.1[a,c]	5.0 ± 5.0
Eosinophils	1.4 ± 0.6[b]	0.9 ± 1.1[b]	1.0 ± 1.1[c]	1.3 ± 0.5[b]	1.9 ± 0.2[d]	7.5 ± 7.5
Basophils	0.2 ± 0.2[c]	0.6 ± 0.5[b]	0.7 ± 0.5[c]	0.6 ± 0.4[c]	0.2 ± 0.0[e]	1.5 ± 1.5

Data are given as mean ± 1 SD.
[a]Value significantly (P < 0.05) different from that at 0 h.
[b]n = 4
[c]n = 3
[d]n = 2
[e]n = 1

Table 4. Glucose, BUN, and creatinine levels (mg/dL) in swine undergoing cardiothoracic surgery

	Time (h) after start of surgery					
	0 (n = 5)	2 (n = 5)	4 (n = 5)	5 or 6 (n = 5)	7 or 8 (n = 4)	Reference values[1]
Glucose	95.4 ± 24.7	127.6 ± 33.9	107.4 ± 28.8	85.6 ± 22.3	41.5 ± 6.2[a]	91.3 ± 24.9
BUN	8.2 ± 0.4	8.6 ± 0.9	9.2 ± 2.5	11.4 ± 1.7[a]	12.5 ± 1.9[a]	16.4 ± 8.2
Creatinine	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.3	1.6 ± 0.5[a]	1.5 ± 1.0	1.6 ± 0.8

Data are given as mean ± 1 SD.
[a]Value significantly (P < 0.05) different from that at 0 h.

(Table 5). Cl^-, P, and CO_2 levels at hours 2 and later did not differ from baseline values (Table 5). In general, electrolyte values in our swine were consistent with reference values, except for lower K^+ at baseline, lower Ca^{2+} at hour 7 or 8, and increased CO_2 at the 2-, 4-, and 5- or 6-h time points (Table 5).[1]

In addition, we noted changes in serum protein levels, including decreased (P < 0.05) total protein at the 2-h and later time points, decreased (P < 0.05) albumin at the 4-h and later time points, and decreased (P < 0.05) globulin at 7 or 8 h, compared with corresponding baseline values (Table 6). Liver enzymes were mostly unchanged: GGT was decreased (P < 0.05) at 4 and 7 or 8 h, but ALP, ALT, and AST were not changed at any time point, compared with corresponding baseline values (Table 6). There was no significant change in bilirubin levels, but cholesterol was decreased (P < 0.05) at the 2-h and later time points, compared with baseline values (Table 6). All baseline values for total protein, albumin, liver enzymes, bilirubin, and cholesterol were comparable to reference values in swine, except for globulin, which was lower than the reference values at all time points (Table 6).[1] Total protein and cholesterol values were lower (P < 0.05) at 2 h and later, and GGT was lower (P < 0.05) at 7 or 8 h, but all other values were consistent with corresponding reference values in swine (Table 6).[1]

Discussion

The goal of the current study was to test the hypothesis that nonsurvival cardiothoracic surgery performed for 8 h in swine by using clean technique would produce no evidence of bacteremia or sepsis. We evaluated clinical parameters, serial blood cultures, and hematologic and clinical chemistry profiles at regular time points during nonsurvival cardiothoracic surgery in swine. Our results support our hypothesis that clean technique is sufficient to avoid bacteremia and sepsis during prolonged cardiothoracic surgery in swine.

Heart rate and body temperature are clinical parameters that typically are altered in cases of sepsis. In this study, heart rate did not change significantly throughout surgery, a finding consistent with a lack of septicemia. In comparison, body temperatures increased consistently during the surgery. However, the average baseline temperature was below the reference range, likely due to loss of body heat during anesthetic induction, transport to the surgery preparation area, and lack of heat and fluid support during preparation for surgery. Once swine were moved to the operating room, heat support and warmed intravenous fluid support were provided. The latest-recorded body temperatures (at the 7- or 8-h time points) were consistent with reference range values, despite prolonged anesthesia and an open thorax, suggesting the effective provision of thermal support during surgery. Therefore, the perceived increase in temperature during surgery was most likely due to a low baseline temperature rather than to perioperative infection.

Positive blood cultures are the classic indicator of bacteremia or septicemia. However, they also can indicate contamination during sample collection. In true cases of bacteremia or septicemia, positive blood cultures obtained during surgery are followed by subsequent positive cultures at later time points. Therefore, our current finding of positive blood cultures (n = 2) only at T4, with no growth in any sample at the 5- or 6- or the 7- or 8-h time points, suggests iatrogenic contamination of the 4-h samples. The main risk of surgical site infection is the patient's endogenous flora,[8] and coagulase-negative staphylococci, especially *S. epidermidis*, are typical nonpathogenic residents of human skin. In a human hospital setting, these bacteria can result in bacteremia associated with indwelling devices or immunosuppression[15] and are often responsible for

Table 5. Electrolyte and CO_2 levels (mEq/L) in swine undergoing cardiothoracic surgery

	Time (h) after start of surgery					
	0 (n = 5)	2 (n = 5)	4 (n = 5)	5 or 6 (n = 5)	7 or 8 (n = 4)	Reference values[1]
Na^+	143.6 ± 1.5	141.2 ± 1.5[a]	140.4 ± 1.9[a]	140.8 ± 2.2[a]	141.3 ± 2.2	145.9 ± 6.7
K^+	4.2 ± 0.2	4.8 ± 0.2[a]	5.6 ± 0.7[a]	6.9 ± 1.5[a]	8.0 ± 1.9[a]	5.5 ± 1.0
Cl^-	102.4 ± 1.8	101.2 ± 1.5	101.6 ± 1.1	102.2 ± 2.2	105.0 ± 2.9	101.8 ± 4.7
Ca^{2+}	10.2 ± 0.4	9.6 ± 0.4[a]	9.4 ± 0.4[a]	9.1 ± 0.5[a]	8.0 ± 0.4[a]	10.4 ± 1.1
P	9.1 ± 0.5	8.4 ± 0.6	8.9 ± 1.2	9.8 ± 1.1	10.0 ± 1.6	7.4 ± 1.9
CO_2	31.2 ± 0.4	31.4 ± 1.8	32.0 ± 2.5	32.4 ± 3.2	27.0 ± 5.7	26.6 ± 1.6

Data are given as mean ± 1 SD.
[a]Value significantly ($P < 0.05$) different from that at 0 h.

Table 6. Protein (g/dL) and liver values in swine undergoing cardiothoracic surgery

	Time (h) after start of surgery					
	0 (n = 5)	2 (n = 5)	4 (n = 5)	5 or 6 (n = 5)	7 or 8 (n = 4)	Reference values[1]
Total protein	5.5 ± 0.3	4.7 ± 0.4[a]	4.4 ± 0.3[a]	4.0 ± 0.4[a]	3.2 ± 0.7[a]	7.1 ± 1.3
Albumin	3.3 ± 0.5	2.8 ± 0.4	2.6 ± 0.3[a]	2.3 ± 0.1[a]	1.9 ± 0.4[a]	3.2 ± 0.9
Globulin	2.2 ± 0.5	1.9 ± 0.5	1.8 ± 0.5	1.7 ± 0.5	1.3 ± 0.4[a]	5.0 ± 1.1
ALP (U/L)	153.2 ± 22.5	136.2 ± 18.8	132.0 ± 20.4	138.0 ± 19.8	126.8 ± 28.7	109.0 ± 67.6
GGT (U/L)	34.8 ± 5.1	28.4 ± 6.8	25.2 ± 6.7[a]	34.8 ± 30.5	23.0 ± 5.4[a]	41.5 ± 10.5
ALT (U/L)	36.4 ± 10.0	31.2 ± 8.1	27.0 ± 8.1	27.2 ± 8.6	26.5 ± 12.0	34.1 ± 12.4
AST (U/L)	29.2 ± 9.1	30.0 ± 7.8	28.0 ± 9.9	30.6 ± 7.7	41.0 ± 11.0	35.3 ± 20.0
Bilirubin (mg/dL)	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.25 ± 0.25
Cholesterol (mg/dL)	77.4 ± 6.9	65.6 ± 6.7[a]	61.6 ± 3.9[a]	58.6 ± 9.9[a]	48.3 ± 11.6[a]	107.8 ± 26.4

Data are given as mean ± 1 SD.
[a]Value significantly ($P < 0.05$) different from that at 0 h.

infections associated with cardiothoracic surgery.[6] In addition, staphylococci are common skin microflora of pigs. For example, skin grafts from Yorkshire and Raceland xenograft donor pigs cultured prior to antibacterial treatment yielded coagulase-negative staphylococci as 70% to 95% of the bacterial population.[21] Another study found 1 to 5 species of staphylococci in 96.9% of mammary skin samples from crossbred sows, and 8 of the 10 *Staphylococcus* species identified were coagulase-negative.[17] Assuming that the positive cultures in our study represent contamination, 2 of 24 cultures (8.3%) were contaminated, representing a contamination rate similar to that reported in human hospitals.[7,23]

The results of a CBC and WBC differential analysis are often useful for diagnosing clinical disease, including sepsis. In the current study, direct counts of RBC as well as the calculated values of Hgb and Hct were decreased at hours 2 and 4 but had recovered to baseline values at hours 5 or 6 and 7 or 8. This pattern of variation is unlikely in a true case of septicemia. The consistency of total WBC counts, representation of individual cell types in the WBC differential analysis, and platelet numbers throughout surgery in the current study further support a lack of systemic infection.

Notable clinical chemistry results included increased BUN and creatinine values at late (hours 5 or 6 and 7 or 8) time points and decreased Na^+ values at early (hours 2, 4, and 5 or 6) time points, but all were within reference ranges. These results indicate that glomerular filtration rate and renal function were maintained throughout anesthesia, and adequate hydration was provided by intravenous fluids.

Glucose at hour 7 or 8 was decreased compared with baseline and was below the reference range. Possible causes of hypoglycemia include starvation, malabsorption, extreme exertion, hepatic insufficiency, sepsis involving a gram-negative or an anaerobic gram-positive bacterium, and various endocrine disorders.[10] However, neither the history of the swine nor the hematologic or clinical chemistry profiles support any of these as causes of low glucose at hour 7 or 8. Two bacterial cultures from 2 separate animals were positive. However, the organisms cultured were *Staphylococcus* species, the time point of positive cultures was different from that of hypoglycemia, and cultures at subsequent time points were negative. Therefore, the hypoglycemia seen at hour 7 or 8 in these pigs was not due to gram-negative or anaerobic gram-positive sepsis. The swine were fasted for approximately 16 h prior to anesthetic induction, suggesting that lack of caloric intake during this time, and during the 7 to 8 h of anesthesia, likely contributed to the hypoglycemia seen at hour 7 or 8. In addition, glucose at the 2-h time point exceeded the reference range and was increased nonsignificantly above baseline. The use of xylazine in these pigs may have had a protective effect on glucose at the earlier time points, maintaining the concentration either within or slightly above reference values, because xylazine may cause transient hyperglycemia in multiple species, including swine.[19] This would explain the initial slight increase in glucose that we saw at the 2-h time point, followed by a gradual decrease at the 4- and 5- or 6-h time points, and then hypoglycemia at the 7- or 8-h time point. In addition, significantly decreased serum glucose in crossbred Kogata Chinese–Clawn–Göttingen minipigs occurred after 90 min of isoflurane anesthesia,[25] indicating that anesthesia may have contributed to the hypoglycemia that we noted in our pigs.

In the current study, levels of Ca gradually decreased over time and were below reference range by the 7- or 8-h time point. Considering that 40% of total serum calcium is protein-bound, primarily to albumin,[11] the gradual decrease in calcium that we noted likely was due to the concurrent gradual decrease

in albumin. Indeed, using standard calculations[11] to adjust for either decreased albumin or decreased total protein corrected the Ca level measured at the 7- or 8-h time point to within the reference range, indicating that no functional hypocalcemia was present.

Serum potassium was increased at all time points and exceeded the reference range by the 7- or 8-h time point. Hyperkalemia can be caused by tissue trauma, such as occurs during surgery, when cells are damaged and intracellular K^+ spills into the extracellular fluid.[20] Hyperkalemia also can result from acidosis,[20] but CO_2 levels in the current study do not indicate the presence of acidosis. Hemolysis may cause pseudohyperkalemia due to release of K^+ from RBC, but hemolysis in the serum samples was noted only infrequently in the current study. Therefore, the most likely cause of the hyperkalemia in our swine was tissue trauma resulting from surgery.

Total protein and albumin were decreased from baseline values at most time points; globulin was significantly decreased from baseline at the 7- or 8-h time point. All 3 protein parameters progressively decreased with time, a pattern that is strongly suggestive of gradual hemodilution due to intravenous fluid administration.

Serum ALP, ALT, AST, and total bilirubin did not differ significantly from baseline values at any time point during surgery and remained within reference ranges for the duration of the experiment. GGT was decreased at 4 and 7 or 8 h compared with baseline values, but only the 7- or 8-h value was below the reference range, and the significance of this finding is unclear. Cholesterol levels in our swine consistently were decreased compared with baseline and reference range values and, similar to glucose concentrations, showed the greatest decrease from hour 5 or 6 to hour 7 or 8. Primary hypocholesterolemia in humans typically is due to genetic disorders of cholesterol absorption, biosynthesis, or metabolism,[22] which were unlikely conditions in these pigs. Secondary hypocholesterolemia may be due to anemia, hyperthyroidism, neoplasia, liver disease, severe stress or illness, malabsorption or malnutrition, acute or chronic infection, chronic inflammation, or various drugs.[22] Our results, as well as the recent husbandry and medical history of the pigs in the current study, do not support the existence of these clinical conditions. Hypocholesterolemia is common in critically ill human patients with serious polytrauma, extensive surgery, serious infection, or protracted hypovolemic shock, but it is unclear whether hypocholesterolemia is a manifestation of or contributor to disease progression.[27] The pigs in the current study underwent a major surgery that may have contributed to hypocholesterolemia, but otherwise they did not experience or exhibit signs of polytrauma, serious infection, or hypovolemic shock. Consistent with previous work in anesthetized crossbred Kogata Chinese–Clawn–Göttingen minipigs,[25] isoflurane anesthesia likely contributed to the decreased cholesterol level in our pigs.

A relationship between length of surgical procedure and incidence of surgical site infection has been identified in humans. Procedures longer than 4 h have been shown to increase the risk of surgical site infection.[9,13,24] However, the pigs in the current study underwent surgical procedures up to 8 h in length with no conclusive evidence of bacteremia or sepsis.

Various limitations of the current study could not be avoided. Clean—rather than strict aseptic—technique was approved for the current study only because the swine were euthanized before recovery from anesthesia. As a result, further follow-up regarding the development of surgical site infection or sepsis was not possible. In cases of surgical site infection, bacteria typically gain entry into tissues at the time of the surgical procedure, but infection may not be visibly or clinically apparent until days or weeks after surgery.[13] Therefore, we cannot prove that clean technique prevented bacterial contamination or infection, only that there was no evidence of bacteremia or sepsis as indicated by serial blood cultures, CBC, and clinical chemistry tests. Cytokine assays and paired blood cultures from multiple sites at each time point might have provided additional data and may have ruled in or ruled out sample contamination but were beyond the scope of this study.

Despite these limitations, the current project addresses a deficit in the laboratory animal medicine literature regarding whether aseptic technique is necessary during prolonged nonsurvival surgeries. We used a prospective study to evaluate standard diagnostic clinical parameters in pigs undergoing terminal cardiothoracic surgery of as long as 8 h and found no physiologic, hematologic, or clinical chemistry trends indicative of sepsis. Blood cultures were positive at only 2 of the 24 time points and were negative at subsequent time points.

In conclusion, the current results contribute important information to the field of laboratory animal medicine by providing evidence that clean technique is sufficient to prevent bacteremia and sepsis in swine undergoing prolonged (maximum 8 h) cardiothoracic surgery.

Acknowledgments

We thank Dr David Rabkin's research team for assistance with sample procurement.

Outstanding technical assistance was provided by veterinary technicians and veterinarians in the Department of Comparative Medicine (University of Washington, Seattle, WA). Funds for this project were generously provided by the Department of Comparative Medicine (University of Washington).

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