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BASIC RESEARCH

CHARACTERIZATION OF AN ANIMAL MODEL OF SEVERE SEPSIS ASSOCIATED WITH RESPIRATORY DYSFUNCTION

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PURPOSE: Pathophysiological studies in humans regarding sepsis are difficult to perform due to ethical and methodological concerns. In this context, animal models of sepsis can be useful to better understand this condition and to test therapeutic strategies. The purpose of this study was to characterize a feasible and clinically relevant model of sepsis in pigs that could be useful for testing different therapeutic interventions.
METHODS: 5 White Large pigs were anesthetized, arterial and pulmonary catheters were introduced, and sepsis was induced by fecal peritonitis. Several biochemical indicators of organ dysfunction and infectious parameters were measured. The pigs were monitored until death, when fragments of organs were removed for pathology. Three animals without peritonitis served as controls and were sacrificed 24 hours after surgery without developing significant changes in organ function.
RESULTS: Septic pigs survived 17 hours on average (range, 16-18 h), and *Escherichia coli* was recovered from blood cultures. They developed a significant decrease in left ventricular work and a nonsignificant reduction in mixed venous oxygen saturation. Respiratory dysfunction was characterized by a decrease in the PaO_2/FiO_2 ratio and respiratory compliance. Pathology of the lungs revealed areas of pulmonary collapse, hemorrhage, pulmonary congestion, and discrete neutrophil infiltrate.
CONCLUSIONS: Fecal peritonitis in pigs is a clinically relevant model of sepsis associated with acute lung injury without direct pulmonary insult. This model may prove to be useful for studying pathogenic aspects of secondary lung injury as well as for validating ventilatory or pharmacologic interventions.

KEYWORDS: Sepsis. Acute lung injury. Pigs. Animal models. Peritonitis.

INTRODUCTION

Sepsis is at present one of the most common and challenging conditions in critical care medicine. The incidence of this disease has increased in the last years, probably secondary to progressive aging of the population, improvements in therapeutic support to immunocompromised patients, and increases in bacterial resistance.[1] Despite improvements in therapeutic support in recent years, the mortality in cases of severe sepsis and septic shock is still extremely high, with numbers between 30% to 60%, depending on the case series.[1–3]

Human pathophysiological studies conceived to identify pathways associated with beneficial effects from therapeutic interventions are frequently hard to perform due to methodological and ethical issues. In this regard, the use of animal models allows investigators to elaborate studies incorporating molecular and cellular techniques that are not suitable to be performed in human beings. Although animal models are subject to critique on the relevance of experimental data regarding the translation to clinical sepsis, they are still one of the best resources for studying the pathophysiology of sep-

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sis.[4–7] Experimental models of sepsis have been used extensively to explore the pathogenesis of sepsis and to generate preclinical data for therapeutic interventions.

The aim of the present study was to develop and characterize an easy, reproducible, and clinically relevant animal model of sepsis with an infectious focus (peritoneum) that resembles the human disease and that may be useful for testing therapeutic interventions. The effects of sepsis on hemodynamics, inflammatory profile, and organic dysfunction are described.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Research Ethics Committee and was performed according to National Institutes of Health guidelines for the use of experimental animals.

Animals and surgical preparation

Eight domestic White Large pigs with body weights of 40 to 45 kg were fasted overnight before the experiment, with free access to water. The pigs were premedicated with midazolam (0.3 mg/kg) and acepromazine, anesthesia was induced with thionembutal (12 mg/kg), and muscular relaxation was induced with pancuronium bromide (0.1 mg/kg). Subsequently, they were submitted to endotracheal intubation and connected to a mechanical ventilator (Evita 2, Drager, Luebeck, Germany) with the following parameters: tidal volume, 8 mL/kg; end-expiratory pressure, 5 cm H_20. FiO_2 was initially set at 30% and subsequently adjusted to keep arterial saturation above 94%. Respiratory rate was set at the level necessary to keep $PaCO_2$ between 35 and 45 mm Hg. Anesthesia was maintained during the surgical period with thionembutal (10 mg/kg/h) and fentanil (5 mcg/kg/h) and muscular relaxation was maintained with pancuronium bromide (0.2 mg/kg/h). After surgery, only thionembutal and pancuronium were maintained in the concentrations described above.

The right jugular external vein was dissected and cannulated for introduction of a pulmonary artery catheter that was guided to the pulmonary artery by pressure curve visualization. The right femoral artery was dissected and cannulated, and a catheter was inserted to continuously measure arterial pressure and to collect arterial samples. The right femoral vein was cannulated with a double-lumen catheter for fluid and drug administration as well as to collect blood samples. A cystostomy was performed, and a vesical catheter was inserted to measure urinary output.

After instrumentation, a midline laparotomy was performed, and the spleen was removed to avoid autotransfusion during shock.[8–10] The descending colon was visualized, a 2-cm incision was performed, and 1.5 g/kg of fecal content were removed. The intestinal incision was sutured, and 2 large-bore catheters were inserted in each flank of the animal. Then, the laparotomy was closed. After instrumentation, the animals were allowed to stabilize for 1 hour.

Experimental protocol

After the stabilization period, 5 animals received 1.5 g/kg of autologous fecal contents diluted in 200 mL of warm (37ºC) saline into the peritoneal cavity through the 2 large-bore flank catheters. The 3 animals that served as controls were injected with 200 mL of normal saline. After sepsis induction, hemodynamic and respiratory variables were collected every hour until death. In control animals, sacrifice with potassium chloride was performed after 24 hours.

The protocol for fluid management included a 24-hour infusion of 1000 mL of 10% glucose with potassium chloride 25 mEq and sodium chloride 75 mEq to keep glycemia and electrolytes at near-normal levels. During anesthesia and the surgical period, each animal received 1500 mL of Ringer's lactate to assure euvolemia before sepsis induction. After the induction of peritonitis, the pigs received volemic expansion (aliquots of 500 mL of Ringer's lactate in 30 minutes) only when any one of the following 3 conditions occurred: central venous pressure values decreased to less than 10 mm Hg; mean arterial pressure decreased to less than 70 mm Hg; diuresis was less than 0.5 mL/kg/h for 1 hour.

Respiratory monitoring

Expired minute volume; tidal volume; respiratory rate; peak, mean, and end-inspiratory pressures; positive end expiratory pressure; end-tidal carbon dioxide concentration; inspiratory oxygen fraction; static compliance; airway resistance; and arterial oxygen saturation were measured continuously throughout the study period.

Hemodynamic monitoring

Mean arterial blood pressure, central venous pressure, and pulmonary artery pressure were measured with Quartz transducers (Edwards Critical Care, Irvine, CA, USA) and displayed continuously on a multimodular monitor (DX 2020, Dixtal, São Paulo, Brazil). Heart rate was measured from the electrocardiogram. Continuous central venous oximetry was measured by spectrophotometry, and continuous cardiac output was measured by thermodilution (Vigilance®, Edwards Lifesciences, Irvine, CA, USA). Central temperature was obtained from the thermistor in the pulmonary artery catheter.

Laboratory, inflammatory, and NO metabolite analysis

Arterial and venous blood samples were collected immediately before induction of peritonitis for measurement of blood gases, arterial lactate, sodium, potassium, chloride, hemoglobin, glycemia, and calcium. The concentrations of these compounds were measured in a blood gas analyzer (ABL 700 Radiometer, Copenhagen, Denmark). Arterial blood gases were determined every 6 hours after induction of peritonitis, and venous blood gases were determined every 12 hours for continuous venous oximetry calibration. Biochemical and hematological tests were performed before and 12 hours after peritonitis was induced and included: blood urea nitrogen, creatinine, bilirubin, and hemogram. Twelve hours after the induction of sepsis, 20 mL of blood were collected aseptically for blood cultures.

Before the induction of peritonitis and at 2, 4, 6 and 12 hours thereafter, blood samples were withdrawn for measurement of interleukin-6 (IL-6) concentration. Cytokine (IL-6) concentration in plasma was measured with a specific commercially available enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer instructions (R&D Systems, Minneapolis, MN, USA).

Serum levels of nitrite/nitrate (nitric oxide (NO) metabolites) were measured before and 12 hours after sepsis by a chemiluminescence technique (Sievers NO analyzer, model 280, Sievers Instruments Inc, Boulder,CO, USA). Briefly, this technique requires reduction of nitrite and nitrate to NO. Nitric oxide reacts with ozone to generate light, which is captured by photomultipliers. To convert nitrate to NO, a reaction with vanadium chloride in hydrochloric acid at 95ºC was performed; to convert nitrite to NO, potassium iodide in acetic acid was used.

Pathology

Immediately after death, small fragments of lung, liver, kidney, heart, and small bowel were removed from all animals and placed in phosphate-buffered saline for subsequent histological analysis. Formalin-fixed tissues were routinely processed and stained with hematoxylin and eosin before examination by an experienced histopathologist who was blinded as to whether the animals were septic or not.

Statistical analysis

Normal distribution of each parameter was ascertained through the Kolmogorov-Smirnov goodness of fit model, and is shown as mean and standard deviation. Within-group and between-group means as well as the time factor interaction were analyzed using 2-way analysis of variance (ANOVA 2-way). The commercially available SPSS 10.0 (Chicago, Il, USA) statistical package software was used, and $P < .05$ was considered statistically significant.

RESULTS

Survival Time

The septic animals survived an average of 17 hours (range, 16-19 h). Control pigs were sacrificed 24 hours after surgery without any signs of hemodynamic or respiratory derangement.

Hemodynamics

Table 1 lists hemodynamic parameters from septic and control animals. As expected, peritonitis induced a significant decrease in mean arterial pressure, as well as an increase in heart rate. Sepsis also induced a significant decrease in left ventricle work and a nonsignificant decrease in mixed venous saturation. Cardiac filling pressures tended to rise (nonsignificantly) in the later phase of the study, a finding that was compatible with myocardial dysfunction and with the high cumulative hydric balance that was necessary to maintain arterial pressure in septic animals, when compared with control pigs. Despite the high positive water balance, the urinary output of septic pigs was significantly reduced when compared to that of control pigs.

Lung Function

Although not associated with direct pulmonary damage, this peritonitis model of sepsis induced acute lung injury, as demonstrated in Table 2. Pulmonary compliance was significantly reduced in both groups of pigs during the course of the study, a finding probably associated with injury in the septic group and the prolonged supine position in the control group. Septic pigs also fulfilled criteria for Acute Lung Injury (ALI), because the PaO_2/FiO_2 ratio decreased to less than 300 in these animals.

Serum chemistry, NO metabolites, and inflammatory markers

Table 3 shows laboratory data, core temperature, and NO metabolites in septic and control animals. Although BUN and creatinine levels did not differ between septic or control pigs, 2 septic animals developed clinical signs of renal dysfunction evidenced by oliguria. Sepsis also caused an increase in serum levels of potassium when compared with control animals at the end of experiment, despite similar amount of

Table 1 - Evolution of hemodynamic parameters in septic and control groups

Variable	Group	Baseline¶	1 hour	3 hours	6 hours	12 hours	Before death	ANOVA*P* value
Heart rate(beats/min)	Septic	102 ± 27	125 ± 26	174 ± 27	172 ± 16 §	146 ± 27 §	139 ± 16 §	< .001*
	Control	122 ± 20	120 ± 18	120 ± 19 ‖	117 ± 14 ‖	108 ± 26 ‖	107 ± 30 ‖	< .001#
Mean ABP(mm Hg)	Septic	111 ± 9	97 ± 9	90 ± 15 §	82 ± 15 §	74 ± 8 §	62 ± 9 §	< .001*
	Control	122 ± 20	120 ± 18 ‖	120 ± 19 ‖	117 ± 14 ‖	108 ± 26 ‖	122 ± 20 ‖	.009#
Mean PAP(mm Hg)	Septic	28 ± 6	27 ± 8	30 ± 4	34 ± 8	34 ± 6	44 ± 7	.47*
	Control	31 ± 5	26 ± 4	29 ± 5	30 ± 3	28 ± 3	38 ± 2	.56#
Cardiac output(mL/min/kg)	Septic	109 ± 54	94 ± 51	72 ± 35	41 ± 23	69 ± 23	71 ± 20	.86*
	Control	118 ± 40	103 ± 26	86 ± 21	87 ± 14	66 ± 3	69 ± 13	.22#
LVW(mL x mm Hg/kg)	Septic	1.2 ± 0.5	0.8 ± 0.5	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	.002*
	Control	1.7 ± 0.5	1.4 ± 0.5	1.1 ± 0.7	1.0 ± 0.4	0.7 ± 0.2	0.7 ± 0.1	.42#
CVP(mmHg)	Septic	12.8 ± 2.4	11.0 ± 3.1	9.8 ± 2.4	10.6 ± 3.6	14.0 ± 4.6	20.4 ± 7.4	.43*
	Control	16.3 ± 2.9	14.0 ± 2.0	14.7 ± 2.5	12.3 ± 1.2	12.3 ± 0.6	14.0 ± 1.0	.91#
P wedge(mm Hg)	Septic	11.4 ± 6.0	8.8 ± 3.7	8.4 ± 3.1	9.4 ± 4.0	14.3 ± 8.5	19.0 ± 3.6	.06*
	Control	17.3 ± 2.1	17.0 ± 2.6	14.7 ± 0.6	13.7 ± 2.3	12.7 ± 1.5	15.3 ± 0.6	> .99#
SVR(mm Hg/mL/min/kg)	Septic	1.1 ± 0.4	1.1 ± 0.4	1.3 ± 0.4	1.4 ± 0.3	0.9 ± 0.4	0.6 ± 0.1	.22*
	Control	1.0 ± 0.3	1.0 ± 0.3	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.5	1.4 ± 0.8	.82#
PVR(mm Hg/mL/min/kg)	Septic	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.3	0.4 ± 0.2	.12*
	Control	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.44
SvO_2(%)	Septic	74 ± 2	70 ± 11	58 ± 8	51 ± 18	52 ± 4	34 ± 13	.11*
	Control	73 ± 4	68 ± 3	64 ± 11	64 ± 7	60 ± 10	59 ± 6	.57#
Diuresis(mL/kg/hour)	Septic	1.3 ± 0.9	0.9 ± 0.4	0.9 ± 0.5	0.6 ± 0.2	1.0 ± 0.9	0.3 ± 0.2	.01*
	Control	2.0 ± 1.2	2.4 ± 1	1.3 ± 0.3	1.6 ± 1.1	1.7 ± 0.8	1.5 ± 1.5	.62 #
Cumulative hydric balance(mL/kg)	Septic	34 ± 22	43 ± 18	62 ± 2	102 ± 19 §	182 ± 37 §	296 ± 55 §	.001*
	Control	48 ± 10	52 ± 13	57 ± 19	64 ± 21 ‖	75 ± 39 ‖	120± 45 ‖§	.001#

ABP: arterial blood pressure; PAP: pulmonary arterial pressure; LVW: left ventricular work; CVP: central venous pressure; SVR: systemic vascular resistance; PVR: pulmonary vascular resistance; SvO_2: mixed venous oxygen saturation
*2-way ANOVA between-group analysis; #2-way ANOVA within-group analysis, Bonferroni's correction for multiplicity was used.
There were no statistical differences in baseline characteristics between the 2 groups (Student *t* test).
‖ $P < .05$ vs septic group (Dunn's test)
§ $P < .05$ vs baseline (Tukey's test)

Table 2 - Evolution of respiratory parameters in septic and control groups

Variable	Group	Baseline	6 hours	12 hours	Before death	ANOVA*P* value
Compliance(mm Hg/mL)	Septic	24 ± 4	22 ± 4	16 ± 2 §	10 ± 4 §	.84*
	Control	21 ± 5	19 ± 2	19 ± 6	13 ± 1 §	< .001#
Resistance(mm Hg/L/sec)	Septic	15 ± 3	15 ± 2	15 ± 4	8 ± 4	< .001*
	Control	7 ± 1‖	7 ± 1‖	8 ± 2‖	9 ± 1	.12#
FiO_2	Septic	0.29 ± 0.02	0.29 ± 0.07	0.37 ± 0.09	0.37 ± 0.09	.12*
	Control	0.27 ± 0.05	0.22 ± 0.02	0.22 ± 0.02	0.24 ± 0.05	.17#
PaO_2/FiO_2(mm Hg)	Septic	380 ± 73	320 ± 44	232 ± 63 §	204 ± 79§	.07*
	Control	418 ± 26	383 ± 45	365 ± 37‖	367 ± 91‖	< .001#
$PaCO_2$(mm Hg)	Septic	40 ± 5	37 ± 3	41 ± 8	43 ± 9	.39*
	Control	48 ± 11	38 ± 7	35 ± 2	37 ± 3	.98#
Respiratory rate(breaths/min)	Septic	21 ± 4	22 ± 4	22 ± 3	22 ± 3	.73*
	Control	21 ± 5	24 ± 0	23 ± 1	21 ± 1	.59#

*2-way ANOVA between-group analysis; #2-way ANOVA within-group analysis, Bonferroni's correction for multiplicity was used.
‖ $P < .05$ vs septic group (Dunn's test)
§ $P < .05$ vs baseline (Tukey's test)

potassium replacement. Septic animals also developed acidosis during the course of the disease, whereas control animals went into alkalosis. A significant finding of our model is that some septic animals developed leukopenia and lymphocytosis (not shown). These results are consistent with severe gram-negative infections, whereas control pigs had leukocytosis that may be explained as part of normal response to surgical stress. Peritonitis also induced coagulation disturbances, evidenced by significant reduction in platelet counts in the sepsis group. As depicted in Table 3, serum levels of nitrite and nitrate did not increase significantly in either group during the course of the study.

Table 3 - Laboratory data from septic and control groups

Variable	Group	Baseline	6 hours	12 hours	Before death	ANOVA*P* value
BUN(mg/dL)	Septic	14 ± 4	———	———	19 ± 6	.63*
	Control	21 ± 12	———	———	15 ± 4	.81#
Creatinine(mg/dL)	Septic	1.1 ± 0.2	———	———	1.3 ± 0.3	.74*
	Control	1.3 ± 0.3	———	———	1.1 ± 0.2	.94#
Sodium(mmol/L)	Septic	135 ± 5	———	———	132 ± 5	.62*
	Control	136 ± 3	———	———	134 ± 8	.48#
Potassium(mmol/L)	Septic	3.6 ± 0.4	———	———	5.0 ± 0.8 §	.04*
	Control	3.7 ± 0.3	———	———	3.7 ± 0.4 ‖	.03#
Calcium(mmol/L)	Septic	2.2 ± 0.4	———	———	2.0 ± 0.2	.049*
	Control	2.6 ± 0.2	———	———	2.4 ± 0.2 ‖	.29#
Chloride(mmol/L)	Septic	103 ± 5	———	———	109 ± 7	.14*
	Control	103 ± 2	———	———	100 ± 4	.67#
Hemoglobin(g/dL)	Septic	11.2 ± 1.2	———	———	11.7 ± 1.6	.75*
	Control	10.8 ± 0.7	———	———	12.6 ± 1.7	.19#
Leukocytes(cells/mL^3)	Septic	12478 ± 4257	———	———	7648 ± 1518	.02*
	Control	12600 ± 2679	———	———	17703 ± 5313 ‖	.95#
Platelets(cells/mL^3)	Septic	333000 ± 51498	———	———	119500 ± 76177 §	.67 *
	Control	245000 ± 69347	———	———	178667 ± 45236	.001#
Glucose(g/dL)	Septic	126 ± 39	———	———	71 ± 23	.07*
	Control	124 ± 51	———	———	85 ± 7 §	< .001#
Lactate(mmol/L)	Septic	2.0 ± 1.1	1.8 ± 0.6	2.5 ± 2.5	2.6 ± 2.5	.56*
	Control	2.7 ± 2.1	1.0 ± 0.3	1.1 ± 0.4	1.2 ± 0.8	.09#
pH	Septic	7.38 ± 0.03	7.36 ± 0.05	7.30 ± 0.12	7.28 ± 0.11	.03*
	Control	7.34 ± 0.10	7.46 ± 0.04	7.53 ± 0.04	7.49 ± 0.02	.14#
SBE(mmol/L)	Septic	-1.0 ± 1.4	-3.7 ± 3.3	-5.5 ± 5.3	-6.0 ± 5.0	.002*
	Control	-0.1 ± 2.1	2.8 ± 3.2 ‖	5.7 ± 3.7‖	4.5 ± 2.8 ‖	.16#
Nitrite	Septic	0.3 ± 0.2	-	0.2 ± 0.1	-	.67*
	Control	0.3 ± 0.2	-	0.1 ± 0.03	-	.81#
Nitrate	Septic	70 ± 84.7	-	39 ± 40.3	-	.70*
	Control	101.7 ± 51.9	-	40.5 ± 26	-	.77#
Core temperature(°Celsius)	Septic	37.1 ± 1.3	38.8 ± 1.5	39.2 ± 1.1	38.0 ± 1.4	.66*
	Control	36.4 ± 1.3	38.1 ± 1.3	38.4 ± 1.1	38.5 ± 1.6	.52#

BUN: blood urea nitrogen; SBE: standard base excess
*2-way ANOVA between-group analysis; #2-way ANOVA within-group analysis, Bonferroni's correction for multiplicity was used.
There were no statistical differences in baseline characteristics between the 2 groups (Student *t* test).
‖ $P < .05$ vs septic group (Dunn's test)
§ $P < .05$ vs baseline (Tukey's test)

Figure 1 demonstrates the time course of plasma IL-6 levels in this model of peritonitis-induced sepsis. Interleukin-6 levels increased significantly in the septic group 4 hours after induction of peritonitis, reached peak levels 6 hours after, and remained high during the time course of the disease, whereas control animals had persistent diminished levels of this cytokine.

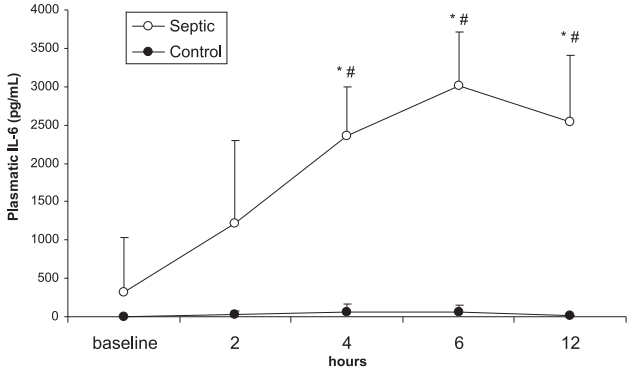

Figure 1 - Time course of plasma concentrations of interleukin-6 (IL-6) in septic and control animals. All values are given as mean ± SD. # $P < .05$ vs. control (Dunn's test); * $P < .05$ vs. baseline (Tukey's test).

Blood cultures

All pigs in the septic group were bacteremic with gram-negative organisms. *Escherichia coli* were recovered from blood cultures of all septic animals. Other organisms isolated in different animals from septic group included *Enterobacter aerogenes, Aeromonas caviae*, *Citrobacter diversus,* and *Streptococcus viridans*. *Escherichia coli* and *Aeromonas hydrophila* were isolated from 2 animals in the control group, and *Pseudomonas fluorescens* and *Streptococcus dysgalactiae* were recovered in blood sample of 1 animal in control group.

Pathology

Histology showed some interesting abnormalities. Neither necrosis nor apoptosis was a major feature. Biopsy from the small bowel of septic animals showed signs of purulent serositis, as well as lymphoid hyperplasia consistent with inflammatory response. Control pigs had no significant alterations. Renal histology revealed no tubular obstruction or necrosis. An isolated finding was the presence of apoptotic tubular cells in only 1 septic kidney. Moderate renal congestion was evidenced in 3 septic animals, but control pigs had a normal histological appearance. Liver samples from septic pigs revealed only congestion, and heart biopsy revealed small foci of subendocardic coagulative necrosis, with discrete inflammatory infiltrate.

The lungs were the organs with the most evident dysfunction evidenced on histology (Figure 2). Macroscopic examination showed extensive zones of pulmonary collapse as well as hemorrhage and pulmonary infarctions (panel A), while control animals had a few regions of collapse restricted to gravity-dependent zones, with the remaining parenchyma appearing normal (panel B). Microscopic examination revealed areas of alveolar collapse, pulmonary congestion, hemorrhage, and foci of discrete neutrophil infiltrate in septic animals (Figure 1, panels C and D), contrasting with the preserved pulmonary structure in control pigs (Panel E). These results demonstrate significant lung compromise from peritonitis, despite an absence of direct lung involvement in septic injury.

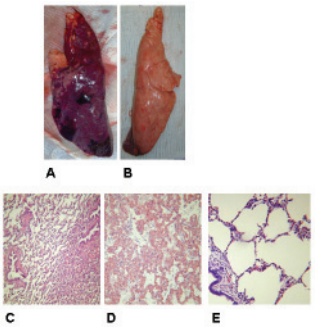

Figure 2 - Gross specimens of lung from septic (A) and control animals (B). Representative lung histology depicting collapse (C) and pulmonary hemorrhage (D) in septic pigs and normal pulmonary parenchyma in control animals (E).

DISCUSSION

We describe in this report a reproducible, clinically relevant, severe sepsis model in anesthetized pigs, characterized by acute lung injury, bacteremia, and organ dysfunction, as well as enhanced inflammatory response (characterized by an increase in serum levels of the cytokine, IL-6).

Animal models of sepsis have been used to study pathogenetic aspects of disease as well as to evaluate therapeutic interventions before the translation of these interventions for human therapy. In this regard, sepsis models have been divided into those with presence or absence of an infectious focus. Models not associated with an infectious focus include endotoxemia and injection of live or killed bacteria, and models with an infectious focus are divided into bacterial inoculation, peritonitis, and fecal sepsis.[11] Endotoxicosis and bacterial infusion models, although more commonly employed, are more prone to critique than models with an infectious focus. This criticism may be due to the fact that endotoxicosis does not replicate common findings of human sepsis, since endotoxin causes sudden and generally transient increases in the inflammatory response.[5] In contrast, the inflammatory stimulus in a true infection, such as pneumonia or peritonitis, tends to develop more gradually and persist over hours or days.[4] Another important characteristic of endotoxicosis is that administration of lipopolysaccharide (LPS), notably in small animals, induces a hypodynamic state that does not resemble the usual pattern noted in human sepsis. In order to circumvent these findings, some different strategies have been developed, such as aggressive fluid resuscitation or continuous infusion of a low dose of LPS. These differences may explain why models not associated with an infectious focus are considered of limited clinical relevance. In this regard, peritonitis models associated with fluid resuscitation are considered the most clinically relevant.[5]

Animal models of peritonitis involve contamination of the peritoneum in a variety of ways and with a multitude of possible contaminants to challenge the host defense mechanisms both locally and systemically. These models include peritoneal soilage induced by cecal ligation and puncture (CLP), fecal pellet implantation, or direct fecal soilage as well as introduction of laboratory-grown bacteria into the peritoneum.[11] Like most clinical situations, peritonitis models are polymicrobial, and the degree of peritoneal contamination can vary widely among the models. This similarity with the clinical picture, although an obvious virtue, is also a major drawback of these models, since the investigator is not able to control the microbiology and the growth of bacteria in the peritoneum in the resulting infection. The inoculation of a standard fecal content, such as we used in our model, can overcome some of these problems, although the precise quantification of bacteria in feces may be a matter of concern.[4]

In our model, sepsis induced a significant compromise in myocardial performance, as evidenced by the significant decrease in left ventricular work; the nonsignificant de-

crease in mixed venous saturation despite aggressive resuscitation is also suggestive. The same hemodynamic pattern had already been described in pig models of peritonitis[6,12] and is similar to under-resuscitated septic shock patterns in humans, which was frequently described in the early periods of disease and was "rediscovered" with the new trials of early goal-directed therapy.[13] We recently described alterations in standard base excess and serum lactate level in such a model[14] This hemodynamic profile is also consistent with the decrease in circulating plasma volume, possibly due to pooling and loss of plasma to the third space, as evidenced by maintenance of hematocrit (data not shown) in septic animals despite the large fluid volumes used for resuscitation.

Respiratory injury was the most evident dysfunction caused by sepsis in our model. Septic animals had a significant decrease in lung compliance and in the PaO_2/FiO_2 ratio, as well as histological evidence of pulmonary injury, such as collapse, hemorrhage, and inflammatory infiltrates. The relationship between sepsis and acute lung injury has been previously reported in large-animal sepsis models.[15] Animals such as sheep and pigs are more prone to turn up with respiratory dysfunction, probably due to the presence of pulmonary intravascular macrophages.[16] These cells are present within the endothelial lining of pulmonary arterioles[17] and, after sepsis stimulus, release cytokines and chemokines that may underlie the pulmonary findings, such as pulmonary hypertension, decrease in lung compliance, and pulmonary edema, as described in our study and those of others.[12,18] It is possible that in our model, part of the increase in alveolar fluid may be caused by the aggressive volume resuscitation performed. However, this pulmonary edema exacerbated by volume replacement is certainly part of septic syndrome in humans, which again emphasizes the similarities between this animal model and clinically relevant sepsis.

The involvement of inflammatory cytokines in our model was assessed by the detection of IL-6 in plasma. We found increased levels of IL-6 in septic animals that peaked 6 hours after bacterial inoculation. Interleukin-6, together with other inflammatory cytokines and chemokines, may underscore some pathophysiological findings presented in septic animals, including sepsis-induced acute lung injury. Unexpectedly, we found no increase in plasma nitric oxide metabolites (NOx) measured after sepsis induction. One might expect that, after sepsis, NOS II activation would generate increased amounts of nitric oxide; since NOx measurements reflect the NO concentration, increased plasma levels of these compounds would be expected. The lack of increase in NOx in septic pigs and dogs has already been described[19,20] and may be related to low production of NO in large animals when compared with rodents, as well as related to fluid resuscitation, which can reduce the residence time of NOx in plasma by enhancing renal plasma flow.[19]

As expected, peritonitis was mainly caused by gram-negative organisms. Contaminants were also isolated from control animals despite all care taken in the surgical period and in culture collection. Pathogenic microorganisms were also recovered in aseptically collected blood cultures from control group. One speculation for this detection after 12 hours of surgery is that total anesthesia and even sham abdominal operation can cause weakened peristalsis resulting in bacterial translocation and bacteremia.[11,21] However, our control group did not demonstrate signs of hemodynamic compromise, respiratory dysfunction, or inflammatory activation 24 hours after surgery.

In conclusion, we characterized a clinically relevant model of severe sepsis in pigs using fecal peritonitis as the focus. Our model demonstrated gram-negative bacteremia, an increase in IL-6 concentrations during the time course of disease, and association with myocardial and respiratory dysfunction. Although no model can be expected to produce all features of sepsis in humans, we anticipate that this model may prove to be useful for testing therapeutic interventions in sepsis.

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RESUMO

PROPOSTA: Estudos sobre sepse envolvendo sua fisiopatologia são difíceis de serem realizados devido a razões éticas e metodológicas. Neste sentido, modelos animais criam oportunidades de estudos para entender a

fisiopatologia e testar estratégias terapêuticas. O objetivo deste estudo foi criar um modelo relevante de choque séptico em porcos para testar e entender diferentes intervenções.

MÉTODOS: 5 porcos da raça "White Large" foram anestesiados e monitorizados com uma linha arterial e um cateter de artéria pulmonar. Uma peritonite fecal foi induzida através de laparotomia. Marcadores de disfunções orgânicas e infecciosos foram mensurados. Todos porcos evoluíram até a morte e amostras de órgãos foram coletadas para exame anátomo patológico. Três animais controles com o mesmo preparo cirúrgico e sem peritonite foram sacrificados após 24 horas de evolução, sem desenvolver mudanças significativas nas funções orgânicas.

RESULTADOS: Os animais séptico sobreviveram na média 17 horas (16 – 18h), e Escherichia coli foi cultivada nas amostras de sangue. Os animais sépticos evoluíram com redução do trabalho de ventrículo esquerdo. A disfunção respiratória foi caracterizada por uma redução na relação PaO_2/FiO_2 e na complacência respiratória. A anatomia patológica dos pulmões revelou colapso pulmonar, hemorragia, congestão e infiltrado neutrofílico.

CONCLUSÕES: A peritonite fecal em porcos é um modelo de choque séptico clinicamente relevante e associada a uma lesão pulmonar sem um insulto direto. Este é um modelo que pode ser utilizado para estudar aspectos fisiopatológicos das lesões pulmonares secundárias, assim como para estudar intervenções ventilatórias ou farmacológicas.

UNITERMOS: Sepse. Lesão pulmonar aguda. Porcos. Modelos animais e peritonite.

REFERENCES

1. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med. 2003;348:1546-54.
2. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001;29:1303-10.
3. Silva E, Pedro MA, Sogayar ACB, Mohovic T, Silva CLO, Janiszewski M, et al. Brazilian Sepsis Epidemiological Study (BASES study). Crit Care. 2004;8:R251-60.
4. Fink MP, Baggs AG. Animal models of sepsis and septic shock. In: Fein AM, et al, editors. Sepsis and multiorgan failure. Williams and Wilkins: New York; 1997. p. 596-613.
5. Parker SJ, Watkins PE. Experimental models of gram-negative sepsis. Br J Surg. 2001;88:22-30.
6. Goldfarb RD, Dellinger RP, Parrillo JE. Porcine models of severe sepsis: emphasis on porcine peritonitis. Shock. 2005;24 (suppl 1):75-81.
7. Rimmelé T, Assadi A, Benatir F, Boselli E, Kaminski C, Arnal F, et al. Validation of a *Pseudomonas aeruginosa* porcine model of septic shock. J Infect. 2006;53:199-205.
8. Hannon JP, Bossone CA, Rodkey WG. Splenic red cell sequestration and blood volume measurements in conscious pigs. Am J Physiol. 1985;248:R293-301.
9. Krejci V, Hiltebrand LB, Erni D, Sigurdsson GH. Endothelin receptor antagonist bosentan improves microcirculatory blood flow in splanchnic organs in septic shock. Crit Care Med. 2003;31:203-10.
10. Marx G, Pedder S, Smith L, Swaraj S, Grime S, Stockdale H, et al. Resuscitation from septic shock with capillary leakage: hydroxyethyl starch (130 KD), but not Ringer's solution maintains plasma volume and systemic oxygenation. Shock. 2004;21:336-41.
11. Kato T, Hussein MH, Sugiura T, et al. Development and characterization of a novel porcine model of neonatal sepsis. Shock. 2004;21:329-35.
12. Kazarian KK, Perdue PW, Lynch W, Dziki A, Nevola J, Lee C-H, et al. Porcine peritoneal sepsis: modeling for clinical relevance. Shock. 1994;3:201-12.
13. Rivers EP, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001;345:1368-77.
14. Park M, Azevedo LCP, Maciel AT, Pizzo VR, Noritomi DT, Cruz Neto LM da. Evolutive standard base excess and serum lactate level in severe sepsis and septic shock patients resuscitated with early goal-directed therapy: still outcome markers?. Clinics. 2006;61:47-52
15. Miwa K, Fukuyama M, Matsuno N, Masuda S, Oyama Y, Ikeda K, et al. Superantigen-induced multiple organ dysfunction in a toxin-concentration-controlled and sequential parameter-monitored swine sepsis model. Int J Infect Dis. 2006;10:14-24.
16. Redl H, Schlag G, Bahrami S, Yao YM. Animal models as the basis of pharmacologic intervention in trauma and sepsis patients. World J Surg. 1996;20:487-92.
17. Morton D, Bertram TA. Isolation and preliminary in vitro characterization of the porcine pulmonary intravascular macrophage. J Leukoc Biol. 1988;43:403-10.
18. Brain JD, Molina RM, DeCamp MM, Warner AE. Pulmonary intravascular macrophages: their contribution to the mononuclear phagocyte system in 13 species. Am J Physiol Lung Cell Mol Physiol. 1999;276:L146-54.
19. Bruins MJ, Lamers WH, Meijer AJ, Soeters PB, Deutz NEP. In vivo measurement of nitric oxide production in porcine gut, liver and muscle during hyperdynamic endotoxaemia. Br J Pharmacol. 2002;137:1225-36.
20. Pastor CM, Hadengue A, Nussler AK. Minor involvement of nitric oxide during chronic endotoxemia in anesthetized pigs. Am J Physiol Gastrointest Liver Physiol. 2000;278:G416-24.
21. Salman FT, Buyruk MN, Gurler N, Celik A. The effect of surgical trauma on the bacterial translocation from the gut. J Pediatr Surg. 1992;27:802-4.