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A Clinically Applicable Porcine Model of Septic and Ischemia/Reperfusion-Induced Shock and Multiple Organ Injury

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Abstract

Background—Although many sepsis treatments have shown efficacy in acute animal models, at present only activated protein C is effective in humans. The likely reason for this discrepancy is that most of the animal models used for preclinical testing do not accurately replicate the complex pathogenesis of human sepsis. Our objective in this study was to develop a clinically applicable model of severe sepsis and gut ischemia/reperfusion (I/R) that would cause multiple organ injury over a period of 48hrs.

Materials and Methods—Anesthetized, instrumented and ventilated pigs were subjected to a ‘Two-Hit’ injury by placement of a fecal clot through a laparotomy and by clamping the superior mesenteric artery (SMA) for 30 minutes. The animals were monitored for 48 hours. Wide spectrum antibiotics and intravenous fluids were given to maintain hemodynamic status. FiO₂ was increased in response to oxygen desaturation. Twelve hours following injury, a drain was placed in the laparotomy wound. Extensive hemodynamic, lung, kidney, liver and renal function measurements and serial measurements of arterial and mixed venous blood gases were made. Bladder pressure was measured as a surrogate for intra-peritoneal pressure to identify the development of the abdominal compartment syndrome (ACS). Plasma and peritoneal ascites cytokine concentration were measured at regular intervals. Tissues were harvested and fixed at necropsy for detailed morphometric analysis.

Results—Polymicrobial sepsis developed in all animals. There was a progressive deterioration of organ function over the 48-hours. The lung, kidney, liver and intestine all demonstrated clinical and histopathologic injury. Acute Lung Injury (ALI) and ACS developed by consensus definitions. Increases in multiple cytokines in serum and peritoneal fluid paralleled the dysfunction found in major organs.

Conclusion—This animal model of Sepsis+I/R replicates the systemic inflammation and dysfunction of the major organ systems that is typically seen in human sepsis and trauma patients. The model should be useful in deciphering the complex pathophysiology of septic shock as it transitions to end-organ injury and thus allow sophisticated preclinical studies on potential treatments.

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INTRODUCTION

Sepsis is the leading cause of death in intensive care units and is defined on a continuum of disorders from sepsis (systemic inflammatory response (SIRS) with suspected infection) to severe sepsis (sepsis + organ dysfunction), septic shock (sepsis + refractory hypotension) and Multiple Organ Dysfunction Syndrome (MODS) [1,2]. Severe sepsis and septic shock carry mortality rates of 25 to 30% and 40 to 70%, respectively [1]. In gross terms, sepsis represents a maladaptive inflammatory, procoagulant, and ultimately immunosuppressive interaction between host and infective pathogen(s). Animal models have elucidated complex signaling and metabolic pathways responsible for the characteristic changes in immune function, coagulation, fibrinolysis, cell death, tissue perfusion, and endocrine function during sepsis. Despite insight into these pathways, and improvements in monitoring, diagnostic modalities and resuscitative and ventilation strategies, novel therapies for sepsis have been remarkably slow to develop. To date, the only consensus approved therapeutic regimes are Early Goal Directed Therapy during the initial stages of disease, and Activated Protein C for severe sepsis [1,3,4].

The inherent difficulties in studying the critically ill population, enormous expense to carry out clinical trials and the complexity of the sepsis spectrum makes animal models essential tools to ultimately improve patient care. But animal models are also at fault for the lack of progress in developing effective sepsis treatments over the last twenty years. Indeed, multiple treatments have shown significant benefits in acute animal models only to worsen the outcome of patients in subsequent clinical trials [5-7]. As a result, many scientists have championed a critical re-evaluation of the criteria necessary for an animal model to truly replicate the complex pathogenesis of human sepsis [8-12]. These criteria include an animal species with similar anatomy and physiology to humans, a clinical setting utilizing standard clinical treatments such as fluid resuscitation, antibiotics and mechanical ventilation, the ability for repeated measurements, and an injury model that accurately reflects the etiology, pathogenesis and complexity of clinical severe sepsis and septic shock [8-12].

The object of this study was to create a model of septic shock with true clinical relevance. To this end, we created a “two-hit” injury model combining intestinal ischemia and reperfusion with intraperitoneal infection. We hypothesized that this model will successfully recreate the progressive and chronic pathogenesis seen in human severe sepsis and septic shock, including a gradual deterioration of organ function and histopathological changes characteristic of sepsis in the intestine, liver and kidney. We believe that this model qualifies as “good evidence” and will serve to generate detailed, reliable and unbiased pre-clinical data in support of treatments that, if successful in this model, would demonstrate a high likelihood of success in human clinical trials [9].

MATERIALS AND METHODS

Anesthesia

Healthy female Yorkshire pigs (n=5, 22-30 kg) were pretreated with glycopyrrolate (0.01 mg/kg, intramuscular), Telazol (tiletamine hydrochloride and zolazepam hydrochloride [5mg/kg, intramuscular]), and xylazine (2mg/kg, intramuscular). A ketamine (3mg/ml) plus xylazine (0.3mg/ml) continuous infusion (3M model 3000 infusion pump) was used to maintain anesthesia at a rate of 100 ml/hr for the duration of the experiment. The rate was adjusted as needed to provide adequate anesthesia. All changes to the rate were recorded.

Tracheostomy and Mechanical Ventilation

An open tracheostomy was performed and the animals connected to a Galileo™ ventilator (Hamilton Medical, Reno, NV). Initial settings were as follows: tidal volume (Vt) 12 cc/kg,

respiratory rate (RR) of 15/min titrated to maintain PaCO₂ within the normal range, FiO₂ of 21% and positive end-expiratory pressure (PEEP) of 3 cmH₂O. Low tidal volume protective mechanical ventilation was not utilized since we did not want to 'protect' the lung with the ventilator but rather measure the development of lung injury if it occurred.

Surgical Preparation

Under sterile conditions, a left carotid artery catheter was placed for blood chemistry and gas content measurements (Roche Inc, Cobas b211) and systemic arterial pressure monitoring. A 4cm right lateral neck incision was made and a veinotomy performed on the right internal jugular vein for placement of a triple lumen catheter for anesthesia, fluid, and antibiotic administration. A right internal jugular Swan-Ganz catheter (7 French) was placed for measurement of pulmonary artery pressure (PAP) and pulmonary artery wedge pressure (PAW), sampling of mixed venous blood gases, and cardiac output (CO) (Agilent, CMS-2001). A Foley catheter was inserted into the bladder for measurement of urine output, collection of urine samples, and was connected to a pressure transducer leveled at mid-axillary line to measure bladder pressure (Pbladder).

Injury

A midline laparotomy was performed and the superior mesenteric artery (SMA) was isolated and clamped for 30 minutes to induce intestinal ischemia. This was confirmed by the loss of the mesenteric pulse as well as the discoloration of the bowel. After 30 minutes the clamp was removed and reperfusion was confirmed by the reappearance of the mesenteric pulse and return of normal color to the bowel. At this point the cecum was brought out of the abdominal cavity and an enterotomy of 2 cm was performed to combine 0.5cc/kg of feces with 2cc/kg of blood obtained from the pig to create a fecal-blood clot. The cecum was returned to its anatomical position, with the enterotomy unclosed, and the clot was implanted into the right lower quadrant of the abdominal cavity. A catheter was placed in Morrison's pouch between the liver and right kidney and brought out through the body wall for collection of peritoneal fluids and sutured to the skin. The abdomen was then closed with 0-0 PDS sutures and the time recorded as T0 (i.e. zero hours following injury). The animals were then followed for twelve hours after injury (T12) at which time the mid-line incision was re-opened for abdominal decompression and allowed to drain passively. Collected ascites were flash frozen for measurement of inflammatory mediators. All of the animals were followed for a total of 48 hours or until time of death.

Fluids and antibiotics

All fluids were warmed to 37°C in a water bath (Precision 280 Series, Thermo Electronic Corp). During the surgical preparation, pigs received a fluid bolus of Lactated Ringers (1 liter IV) over 30 minutes. Following T0 measurements broad-spectrum antibiotics (Ampicillin 2 grams IV [Bristol Myers Squibb, Princeton, NJ] and Flagyl 500mg IV [Baxter, Deerfield, IL]) were delivered over 15 minutes. This antibiotic regimen was repeated at 12, 24 and 36 hours post injury. An intravenous infusion of Lactated Ringers was administered at a rate necessary to maintain adequate volume status determined by urine output (UOP) and mean arterial pressure (MAP). Volume status was deemed inadequate if UOP decreased to less than 0.5cc/kg/hr or if MAP decreased to less than 60 mmHg. All fluids infused or withdrawn were recorded to analyze fluid balance.

Ventilator Adjustments

If arterial desaturation occurred (SaO₂ below 92%) FiO₂ was increased to maintain oxygenation. If 100% FiO₂ did not maintain adequate oxygenation, PEEP was increased (maximum PEEP allowed was 15 cmH₂O) in 2 cmH₂O increments until oxygenation was

adequate or hemodynamics were comprised. If the animal triggered ventilations while fully anesthetized, Pancuronium bromide (0.1mg/kg IV) was given to control breathing.

Hemodynamic measurements

ECG monitoring, pulse oximetry, mean arterial pressure (MAP), central venous pressure (CVP), pulmonary artery pressure (PAP), and pulmonary artery wedge pressure (PAW) were measured (Agilent, CMS-2001™ System M1176A with Monitor M1094B, Boebingen, Germany) using Edwards transducers (Pressure Monitoring Kit PXMK1183, Edwards Lifesciences). Cardiac output (CO) was measured by thermodilution (Agilent, CMS-2001™ System M1176A, Boebingen, Germany). Three separate boluses of cold solution (Dextrose 5% and Sodium Chloride 0.45%) were injected at end-expiration and the average of the three measurements was recorded. Physiologic measurements were made hourly (T0-T48).

Pulmonary Function Measurements

Pulmonary parameters (respiratory rate (RR), peak airway pressure (Pip), mean airway pressure (Pm), plateau pressure (Pplat), tidal volume (Vt), positive end expiratory pressure (PEEP), auto PEEP, expiratory minute volume (EMV), and static compliance (Cstat) were measured or calculated by the Galileo™ ventilator (Hamilton Medical, Reno, NV) at baseline and every hour following injury for 48 hours. Lung function parameters were measured hourly (T0-T48).

Kidney Function

Kidney function was assessed by measuring blood creatinine (Clinical Pathology Department at Upstate Medical University using standard procedures) and BUN (Roche Cobras b221, Roche Diagnostics, Indianapolis, IN) levels at Baseline, every hour for the first 6 hours (BL, T0-T6), and every 6 hours thereafter. UOP was recorded hourly and samples of urine were flash frozen and T0, T12, T36 and T48 for measurement of protein concentration.

Liver Function

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, albumin and total protein were measured by the Clinical Pathology Department at Upstate Medical University using standard procedures. Coagulation parameters (see below) were also measured as indicators of synthetic function of the liver.

Blood Chemistry

Measurement of blood gases and chemistries were made with a Roche Blood gas analyzer (Cobras b221) at Baseline, every hour for the first 6 hours (BL-T6), and every 6 hours thereafter following injury. Both arterial and mixed venous samples were measured for: pH, pCO₂, pO₂, SO₂, hematocrit, hemoglobin, sodium, potassium, chloride, ionized calcium, glucose, BUN, and lactate.

Coagulation parameters

Prothrombin time (PT), international normalization ratio (INR), and activated partial thromboplastin time (PTT) was measured by the Clinical Pathology Department at Upstate Medical University using standard procedures.

Circulating Leukocytes and Platelets

A complete blood count (CBC) with differential including the White Blood Cell (WBC) and platelet count was done by the Clinical Pathology Department at Upstate Medical University using standard procedures.

Inflammatory Mediators

Plasma—Blood was drawn, placed in sodium citrate tubes and spun at 3500 RPM at 15° C for 10 minutes. The plasma was drawn off and snap-frozen in liquid nitrogen for inflammatory mediator analysis.

Peritoneal Fluid (Pfluid)—20ml of saline was injected into the catheter placed in the peritoneum and aspirated back into the syringe 1 minute later. The aspirate (saline plus ascites) was put into blue-topped tubes and spun at 3500 RPM at 15°C for 10 minutes. The supernatant was drawn off and snap frozen for inflammatory mediator analysis.

Bronchoalveolar Lavage fluid (BALF)—At necropsy, the right middle lobe was lavaged with 60ml of normal saline (3 injections of 20 ml flushed into the right middle lobe bronchus and aspirated out) and the volume collected was recorded. The BALF was spun for 10 minutes at 3500 RPM at 15°C for 10 minutes. The supernatant was drawn off and snap frozen for inflammatory mediator analysis.

Mediators Sampled—Inflammatory mediators were measured in *plasma* and *peritoneal fluid* throughout the study and in the *BALF* at necropsy. Tumor necrosis factor alpha (TNF- α), interleukin (IL)-8, IL-6, IL-1 β , IL-12, transforming growth factor beta (TGF- β) (R&D Systems, Minneapolis, MN), IL-10 (Immuno-biological laboratories, Minneapolis, MN), were measured using pig specific ELISA assays according to the manufacturer's assigned specifications. Endotoxin levels were assessed using an end point chromogenic LAL assay (Lonza Group, Ltd. Basel, Switzerland). Lastly, blood was collected in standard vials for aerobic and anaerobic bacteria identification. Mediators sampled in plasma are presented as pg protein per milliliter of plasma. Mediators in Pfluid and BALF were normalized to the total protein present in the sample and presented as pg/ng.

Necropsy

An addendum describing the detailed rationale histological analysis accompanies this manuscript in the supplemental data section.

Lung Histopathology—The heart and lungs were removed en bloc. Gross photographs were made after the lungs were inflated to peak airway pressure of 25cmH₂O. The heart was then removed and the bronchus to the right middle lobe was exposed. The right mainstem bronchus was clamped, the left lung was filled with 10% neutral buffered formalin, and the trachea was clamped. The lung was then immersed in formalin for a minimum of 48hrs before processing for histology. Specimens of dependent lung areas were obtained by measuring 3 cm medial from the aortic groove and making a longitudinal section. Two samples were removed from the most medial section 3cm from the distal tip of the lung.

Kidney Histopathology—The organ was divided along the central axis and specimens of the cortex and adjacent medulla were obtained and fixed in 10% buffered formalin for a minimum of 48hrs.

Intestine Histopathology—Sections of proximal (10cm from the gastric pylorus), mid (estimated mid-jejunum), and distal (10cm from ileocecal junction) small intestine were excised and fixed in 10% buffered formalin for a minimum of 48hrs.

Liver Histopathology—A 3cm section of liver was harvested from the center of the left lobe and fixed in 10% buffered formalin.

All sections were stained with hematoxylin and eosin. Each slide was appraised microscopically and representative histological characteristics were photographed at low, medium, and high magnification (4x, 10x and 40x objectives) (see Additional Files).

Edema Measurement by Wet Dry Weight Ratio (W/D)

Organ edema was measured using the W/D ratio = (wet weight/dry weight) for all 4 organs. Tissue from the lung, intestine, liver, and kidney was excised, minced, immediately weighed and placed in an oven at 60°C and allowed to dry. Dry weight was determined when the weight did not change over a 24 hr period. The wet/dry weights from unpublished historic naïve control animals (n=4) were used as comparison.

Statistics

All data were expressed as mean $\pm$ SE. A repeated measures ANOVA was used to determine differences over time. A student's t-test was used to assess differences in the wet/dry weight ratio of experimental subjects compared to historical controls. Significance was assumed if the probability of the null hypothesis was less than 5% ($p < 0.05$). All analyses were performed on version 5.0.1.2 of JMP (SAS Institute, Cary, NC).

Vertebrate Animals

Experiments described in this study were performed in adherence with the National Institutes of Health guidelines for the use of experimental animals in research. The Committee for the Humane Use of Animals at our institution approved the protocol.

RESULTS

There were no measurable co-morbidities in any animal at the beginning of the experiment. Bacteremia and polymicrobial sepsis was noted in all animals with positive blood cultures for *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. Mortality before T48 was 60%.

Table 1 summarizes the changes in hemodynamic and lung function. Hypotension and hypodynamic shock was evidenced by significant decreases in mean arterial pressure and cardiac output. Acute lung injury (ALI) developed as defined by consensus conference definition with a P/F ratio below 300 without evidence of left ventricular failure [13]. Lung injury was also demonstrated by significant increases in Ppeak, Pplat and decreased Cstat, and confirmed by histological assessment showing congested capillaries and interstitial granulocytic infiltration (See Histology Addendum). Focal alveolar atelectasis with dilated alveolar ducts and alveolar spaces contained fibrinous deposits suggestive of proteinaceous infiltrate (See Histology Addendum). Pbladder rose to greater than 20 mmHg with evidence of lung failure, indicating the development of ACS according to consensus conference definition [14]. The lung wet-to-dry ratio was significantly higher than our historic naïve controls (Figure 2). No macroscopic indications of barotrauma were observed.

Table 2 shows changes in blood composition over the course of the study. There were severe declines in hemoglobin, platelets, total protein and albumin. Percent hematocrit showed

statistically significant changes and WBC fluctuated considerably over the course of the study but did not change significantly with respect to time.

Tables 3-4 summarize the changes in kidney and liver function and coagulation. BUN significantly increased over the course of the study and creatinine doubled over the course of experiment, indicative of acute renal injury. The average hourly urine output dropped significantly over the course of the study despite fluid resuscitation. This could be due to a continual rise in intra-abdominal pressure assessed by bladder pressure, which reached values over 20mmHg. Histopathologic exam of the kidneys showed areas of early cortical tubular atrophy accompanied by interstitial and perivascular edema (See Histological Addendum). There was also loss of tubular architecture with epithelial sloughing, but no injury was seen in the glomeruli (See Histological Addendum). Similar, but less severe injury was noted in the renal medulla. The W/D ratio of the kidney was not significantly different than historic controls (Figure 2). Aspartate aminotransferase (AST) increased but without reaching significance. No significant changes were seen in bilirubin or alkaline phosphatase levels. International Normalized Ratio (INR), prothrombin time (PT) and partial thromboplastin time (PTT) rose to noteworthy levels from the clinical perspective but were not statistically significant. Histopathologic assessment of the liver unveiled conspicuous interstitial edema in the connective tissues of the portal areas and lobular septa, and heavy leukocyte infiltration was frequently observed in these edematous areas. Hepatic lobules were commonly marked by peripheral congestion of the sinusoids and distinct paracentral necrosis (Histological Addendum). Loss of cellular architecture was focal and limited to the proximity of the central vein. The W/D ratio was not significantly different than the historic control (Figure 2).

Without reliable clinical indications of intestinal injury, histopathology is the strongest evidence available to assess disease state (Histological Addendum). Pathology was most prominent in the mucosa, with loss of the surface epithelium, flattening of denuded villi, and sloughing of the lamina propria onto the intestinal lumen. There was also a high incidence of congestion of small blood capillaries in the upper compartment of the mucosa, which was suggestive of hypoxia associated with poor blood flow through the end-capillary bed. The submucosa was grossly edematous and marked by prominently dilated lymph vessels. The cellularity and edema present in the serosa were typical of acute peritonitis (Histological Addendum). The W/D ratio was significantly greater than that of historic control animals (Figure 2).

Table 5 summarizes the blood chemistry data. Arterial pH showed significant changes over time, as did arterial lactate, which rose sharply during the acute phase of injury, returned to normal with resuscitation, then rose again at the end of the study. Systemic oxygenation, as assessed by venous oxygen saturation (SvO₂) significantly decreased. Potassium significantly increased over the course of the study. Decreases were seen in sodium and chloride but they were not significant. Blood glucose levels also decreased throughout the experiments.

Table 6 and Figure 3 summarizes the inflammatory mediator data in the ascites and plasma. A significant change in several cytokines was observed in the peritoneal ascites fluid (Pfluid). TNF- α , IL-1 β , IL-6, IL-8 and IL-12 had significantly higher levels at the end of the study as compared with baseline values. Systemically, TNF- α and IL-1 β were significantly elevated and IL-12 significantly decreased. TNF- α , IL-1 β , IL-6, IL-8, IL-10, and IL-12 were all present in the BALF.

DISCUSSION

Although animal models have been indispensable to advancing our understanding of the sepsis spectrum, the distinction must be made between experimental models that are not clinically applicable, and those that bear clinical relevance and are therefore “translatable” to human trials. Multiple preclinical studies of potential sepsis treatments have shown promising results in acute animal models, but were ineffective or even increased mortality when tested in human clinical trials. The likely explanation for this discrepancy is that the animal models used did not replicate the complex pathogenesis of human sepsis and thus the drugs tested were not treating the same disease that occurs in humans [5-7]. Multiple authors have commented on the necessary components of an ideal animal model of sepsis [8-12,15-17] (Table 7). Our model fulfills most of these criteria, utilizing an animal with similar anatomy and physiology to humans, inclusion of standard clinical procedures (e.g. fluid resuscitation, mechanical ventilation, antibiotic therapy), a protracted time course that mimics clinical reality, and a relevant etiology. Most importantly, our results are consistent with the systemic inflammation and multiple organ injury that are the hallmarks of sepsis pathophysiology:

Pulmonary

Acute lung injury (ALI) was evidenced functionally by a significant decrease in static compliance and P/F ratio with an increase in peak and plateau pressures. Histopathology was consistent with acute lung injury and there was a significant increase in lung water as compared with Controls. The alveolar collapse, lymphocytic accumulation, and hyaline membrane formation are similar to autopsy findings in human patients that died from severe sepsis and septic shock [18]. The presence of TNF- α , IL-1 β , IL-6, IL-8, and IL-10 in BALF is also consistent with findings in human ARDS patients [19-21]. Combined these data demonstrate that this model causes an insidious onset of acute lung injury typical of ARDS and is consistent with the known human literature [22,23].

Renal

There was a rise in both BUN and creatinine, consistent with human septic shock; a rising creatinine level is a known late manifestation of acute renal injury in human sepsis [24]. The delayed renal (as compared to lung) dysfunction was expected and typical of the sequence of organ injury that occurs in human sepsis. Fewer inflammatory cells reside in the kidney and thus parenchymal cell damage secondary to inflammation is minimal, or protracted [25]. Urine output in our animals dropped precipitously early in the study and remained considerably lower than baseline even with fluid resuscitation. A similar time course is seen in septic humans where early oliguria is the result of reduced renal perfusion and continues as a result of evolving renal injury [24]. Renal histopathology was limited mainly to the convoluted tubules of the cortex, along with some interstitial and perivascular edema; however, the glomeruli were not affected. Minimal renal histopathology is typical in patients with septic shock and demonstrates that renal histopathologic injury does not reflect the severity of the kidney injury [26]. The concept of “cell stunning” has been used to describe this phenomenon and perhaps represents a shift towards a more perfunctory state of cell function in response to sepsis [27]. Thus, our clinical, inflammatory, and histopathologic findings demonstrate the similarity in both injury and time course between our model and acute renal failure seen in human septic shock [26-28].

Liver

Our injury model produced only moderate changes in liver synthetic function and moderate clinical injury as measured by AST/ALT. There was a significant decrease in serum albumin and total protein and an elevated AST and INR. These findings are similar to those in the

first phase of liver dysfunction seen in human septic shock [29]. This *first phase* of primary hepatic dysfunction in sepsis is a result of hepatosplanchnic hypoperfusion and can be blunted or reversed with adequate fluid support [29]. The elevated serum lactate level at the final reading may be due to this primary hepatic dysfunction, resulting in decreased lactate clearance. The increase in INR suggests a diminished hepatic production of coagulation factors. Reduced plasma albumin concentration may be due to decreased liver synthesis or loss due to leakage of plasma proteins into the interstitium secondary to increased capillary permeability [24,30]. Minimal histopathology was seen in the liver, similar to septic patients where hepatic cell injury and liver dysfunction are common, however histopathologic liver damage has been shown to be limited and nonspecific [18]. Thus, our findings are consistent with pathology seen in septic patients, typically showing little inflammation with prominent Kupffer and endothelial cells, and centriacinar necrosis [18]. In summary, the liver dysfunction present in our animal model closely represents the findings seen in human patients undergoing currently accepted fluid resuscitation regimens.

Inflammatory Mediators, and Coagulation

Overall, the cytokine response seen in our study was consistent with that seen in human septic shock. The patterned rises in TNF- α , and IL-6, and the decline in IL-12, mirrored the patterns documented in patients with severe sepsis and septic shock [31-34]. In addition, IL-10 was detectable in peritoneal fluid and BALF, consistent with results from clinical trials demonstrating the immunosuppressive phase of septic shock associated with organ injury [33,35].

Cytokines are also implicated in the coagulation abnormalities and vascular endothelial activation that play a major role in the development of organ dysfunction [36]. Cytokine-induced consumption of anticoagulant proteins and suppression of the fibrinolytic system during sepsis results in deposition of fibrin clots and microcirculatory dysfunction [31,37,38]. We observed evidence of consumptive coagulopathy from laboratory results showing prolonged PT, PTT and elevated INR, as well as histopathological evidence of microthrombosis in all organs. Furthermore, activated platelets participate in the formation of microvascular clots, provide a surface for further coagulation activation, and release inflammatory mediators propagating the inflammatory response [39]. The resultant consumption of platelets likely contributes to the marked thrombocytopenia commonly seen in critically ill and septic patients [39]. All the animals in our model had a significant precipitous decrease in their platelet count throughout the study.

Gastrointestinal

Currently there is no way to measure acute intestinal dysfunction secondary to septic shock at the bedside. However, at necropsy we found significant intestinal edema, a large volume of ascites and extensive histopathologic injury, characterized by denudated villi and epithelial sloughing. Recent work by Malbrain and De Laet has stressed the concept of the gut as a 'motor' of organ injury secondary to septic shock and cautioned about being deterred from this idea because of the difficulty assessing gut function [40]. We demonstrated a significant increase in inflammatory peritoneal fluid and intestinal edema, enough to cause ACS, which likely contributed to injury in other organs. Thus, our model provides evidence of acute intestinal injury that likely contributes to worsening prognosis for severe sepsis and septic shock patients. Shah et al recently demonstrated that a combination of hemorrhagic shock, mesenteric venous hypertension and crystalloid resuscitation lead to the development of ACS in a clinically relevant porcine model [41]. This was the first reported physiologic animal ACS model; our model is the second to create ACS in an etiologically relevant manner, via sepsis and gut ischemia/reperfusion.

Summary

We have developed a clinically accurate, chronic animal model of septic shock that replicates the dysfunction seen in the major organ systems of humans being treated for sepsis. The time course of organ dysfunction was also typical of sepsis pathogenesis in humans. The combination of infected clot plus ischemia/reperfusion injury simulates the two injuries most likely to cause shock in humans (i.e. sepsis and trauma). The model fits most of the criteria necessary for a clinically applicable animal model that will yield 'good evidence' that a treatment effective in this model will also be effective in humans.

Critique of our model

Although our model closely parallels human septic shock, it has several limitations. Only female pigs were used in our study such that possible gender differences may alter our results. Clinical studies have shown a lower incidence of severe sepsis and septic shock in females along with a significantly better prognosis [42]. Use of all males instead of females would have a similar problem in that the septic response may be magnified. Future studies may consider randomizing both males and females into the study groups. Only healthy adolescent pigs were used, but epidemiologic studies have shown sepsis occurring at a mean age of approximately 65 years [43]. In addition, this study does not reproduce the co-morbidities, such as diabetes and COPD that may complicate the clinical course of human septic shock. Logistically it would be extremely difficult to conduct a controlled experiment with older, co-moribund animals due to the inherent variability of the animals at the beginning of the experiment. Lastly, we did not include naïve control, surgery only control, and single injury groups (i.e. fecal clot only, ischemia/reperfusion only) in this manuscript. This study is part of a larger study in which control and single injury groups will be included in the analysis. We felt that the data presented was compelling enough to be disseminated as a translational model of sepsis and trauma induced shock, due to the similarities with human pathophysiology, and its broad potential for application.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Kaplan Meier survival curve showing premature death (prior to T48) in three of the five animals for a mortality rate of 60%.

Figure 2. Organ edema. Wet-to-dry ratio of the lung, liver, kidney, and intestine from septic animals (open boxes) and historic controls (black boxes). Data mean $\pm$ SEM. * = $p < 0.05$ vs. historic controls.

Figure 3. Plasma and Peritoneal Fluid (Pfluid) cytokine concentration. A. Plasma TNF- α (open triangles), IL-1 β (+), IL-12 (open circles), IL-10 (*). B. Plasma IL-6 (open diamonds), TGF- β (x). C. Peritoneal Fluid TNF- α , IL-12, IL-10, TGF- β . D. Peritoneal Fluid IL-8 (open squares), IL-6, IL-1 β . Data expressed as mean $\pm$ SEM. § = $p < 0.05$ by time following RM ANOVA. Panels grouped by concentration, with left side panels (A and C) representing a lower concentration and right side panels (B and D) representing higher concentrations.

Table 1

Hemodynamic and Pulmonary Function

	BL (n = 5*)	T6 (n=5*)	T12 (n=5*)	T24 (n=3*)	T36 (n=2*)	T48 (n=2*)	p<0.05
HR	131.4 ± 7.8	83.4 ± 5.5	75.8 ± 7.9	71.3 ± 7.2	77.5 ± 4.5	76.0 ± 8.0	§
MAP	122.4 ± 4.9	69.4 ± 3.2	66.4 ± 3.7	62.0 ± 1.2	61.5 ± 1.5	51.0 ± 8.0	§
PAP	26.2 ± 1.59	27.0 ± 2.2	29.8 ± 2.6	24.7 ± 2.7	26.0 ± 3.0	31.5 ± 4.5	
CVP	8.8 ± 1.2	6.6 ± 1.2	9.6 ± 1.0	8.0 ± 1.7	7.0 ± 1.0	12.0 ± 1.0	
CO	4.61 ± 0.63	2.74 ± 0.67	1.76 ± 0.22	1.72 ± 0.18	1.86 ± 0.23	1.81 ± 0.11	§
Pbladder	11.2 ± 1.2	12.0 ± 1.9	21.4 ± 2.8	17.0 ± 2.5	18.0 ± 3.0	21.0 ± 3.0	
Temp	36.02 ± 0.42	35.1 ± 0.44	35.8 ± 0.33	35.13 ± 0.12	35.8 ± 0.0	35.8 ± 0.05	
Ppeak	18.2 ± 1.0	19.6 ± 1.0	21.0 ± 1.0	25.3 ± 3.5	22.5 ± 0.5	30.5 ± 3.5	§
Pplat	16.6 ± 0.8	17.8 ± 1.0	19.8 ± 0.8	22.7 ± 2.9	21.0 ± 0.0	28.5 ± 3.5	§
Pmean	6.7 ± 0.5	6.9 ± 0.4	7.1 ± 0.2	8.2 ± 1.4	7.6 ± 0.6	12.0 ± 1.0	
Cstat	24.6 ± 0.6	23.2 ± 0.9	20.8 ± 1.8	19.9 ± 2.3	19.5 ± 0.9	13.9 ± 0.5	§
PaCO ₂	29.3 ± 1.9	30.2 ± 2.9	28.6 ± 3.1	30.6 ± 4.0	35.3 ± 6.4	30.2 ± 2.5	
PaO ₂	110.23 ± 12.03	109.4 ± 6.32	130.23 ± 26.37	205.27 ± 28.3	147.27 ± 55.57	149.15 ± 19.65	§
P/F	524.9 ± 57.3	521.0 ± 30.1	503.2 ± 29.8	488.23 ± 43.3	281.6 ± 123.8	233.6 ± 104.1	§

MAP=mean arterial pressure (mmHg). PAP=pulmonary artery pressure (mmHg), CVP=central venous pressure (mmHg), CO=cardiac output (L/min), Pbladder=bladder pressure (mmHg), Temp=core temperature (C°), Ppeak=peak airway pressure (cmH₂O), Pplat=plateau pressure (cmH₂O), Pmean=mean airway pressure (cmH₂O), PaCO₂=arterial carbon dioxide partial pressure (mmHg), PaO₂=arterial oxygen partial pressure (mmHg), P/F=PaO₂/fraction of inspired oxygen (FiO₂). Data are expressed as mean ± SEM.

§ =p<0.05 following RM ANOVA

Table 2

Blood Composition

	BL (n = 5*)	T6 (n=5*)	T12 (n=5*)	T24 (n=3*)	T36 (n=2*)	T48 (n=2*)	p<0.05
Hgb	11.22 ± 0.34	12.86 ± 0.4	11.44 ± 0.48	10.68 ± 0.41	8.83 ± 2.32	9.70 ± 0.10	§
Hct	34.50 ± 0.93	39.68 ± 1.26	34.70 ± 1.42	32.54 ± 1.44	27.17 ± 7.26	30.15 ± 0.15	§
WBC	12.66 ± 1.79	7.62 ± 2.58	8.2 ± 3.14	7.76 ± 2.54	8.10 ± 3.96	6.55 ± 1.65	
Platelets	400.0 ± 56.1	349.5 ± 47.1	168.7 ± 16.6	188.6 ± 46.7	155.7 ± 70.2	133.0 ± 48.0	§
Total Protein	5.4 ± 0.19	3.54 ± 0.23	2.82 ± 0.06	2.38 ± 0.16	2.37 ± 0.20	2.15 ± 0.15	§
Albumin	3.66 ± 0.14	2.44 ± 0.07	1.94 ± 0.06	1.68 ± 0.12	1.37 ± 0.23	1.25 ± 0.15	§

Hgb=hemoglobin, Hct=hematocrit, WBC=white blood cell count ($\text{K}\mu\text{L}^{-1}$), Platelets ($\text{K}\mu\text{L}^{-1}$), Total Protein (g dL^{-1}), Albumin (g dL^{-1}). Data are expressed as mean ± SEM.

§ = p<0.05 following RM ANOVA

Table 3

Kidney Function

	BL (n = 5*)	T6 (n=5*)	T12 (n=5*)	T24 (n=3*)	T36 (n=2*)	T48 (n=2*)	p<0.05
UOP	126.0 ± 23	30.6 ± 7.7	54.0 ± 25.3	70.3 ± 30.1	60.0 ± 30.0	31.5 ± 11.5	
BUN	8.52 ± 0.65	10.81 ± 0.82	12.58 ± 1.62	16.77 ± 2.82	24.37 ± 5.43	33.0 ± 5.0	§
Creatinine	1.00 ± 0.0	1.00 ± 0.10	1.07 ± 0.12	1.35 ± 0.45	1.65 ± 0.65	2.10 ± 0.80	

UOP=urine output (ml/hr), BUN=blood urea nitrogen (mg dL⁻¹), Creatinine (mg dL⁻¹). Data are expressed as mean ± SEM.

§=p<0.05 following RM ANOVA.

Table 4

Liver Function Panel and Coagulation

	BL (n = 5*)	T6 (n=5*)	T12 (n=5*)	T24 (n=3*)	T36 (n=2*)	T48 (n=2*)	p<0.05
AST	34.0 ± 5.9	32.0 ± 6.3	41.6 ± 7.9	109.6 ± 54.0	41.7 ± 0.7	114.5 ± 77.5	
ALT	35.2 ± 3.4	26.2 ± 2.3	23.8 ± 2.8	25.6 ± 3.1	19.7 ± 6.6	27.0 ± 5.0	
ALK PHOS	208.8 ± 25.8	164.4 ± 21.8	168.0 ± 19.8	179.8 ± 28.1	175.7 ± 64.0	205.0 ± 26.0	
PT	13.22 ± 0.10	14.66 ± 0.53	15.76 ± 0.49	18.12 ± 1.10	28.50 ± 11.42	19.35 ± 2.15	
PTT	43.88 ± 5.22	39.30 ± 3.90	40.80 ± 4.77	32.32 ± 3.88	32.03 ± 6.09	22.80 ± 1.30	
INR	0.998 ± 0.009	1.14 ± 0.5	1.25 ± 0.48	1.49 ± 0.11	2.66 ± 1.28	1.63 ± 0.23	

AST=aspartate aminotransferase (U L⁻¹), ALT=alanine aminotransferase (U L⁻¹), ALK PHOS=alkaline phosphatase (U L⁻¹), PT=prothrombin time (sec), PTT=partial thromboplastin time (sec), INR=international normalization unit. Data are expressed as mean ± SEM.

Table 5

Blood Chemistry

	BL (n = 5*)	T6 (n=5*)	T12 (n=5*)	T24 (n=3*)	T36 (n=2*)	T48 (n=2*)	p<0.05
pH	7.483 ± 0.020	7.412 ± 0.019	7.473 ± 0.026	7.500 ± 0.020	7.366 ± 0.088	7.404 ± 0.05	§
Lactate	2.75 ± 0.13	6.23 ± 0.49	1.93 ± 0.38	1.77 ± 0.23	4.67 ± 2.47	3.95 ± 1.75	§
SvO ₂	81.6 ± 10.4	63.4 ± 3.5	46.3 ± 4.3	44.8 ± 3.5	53.0 ± 6.7	44.2 ± 8.4	§
Glu	100.5 ± 15.4	90.0 ± 10.8	69.3 ± 2.9	48.3 ± 3.8	44.0 ± 12.1	49.5 ± 13.5	§
Na ⁺	149.9 ± 5.0	141.7 ± 1.9	143.7 ± 2.0	139.2 ± 1.4	142.3 ± 1.6	137.1 ± 0.1	
K ⁺	3.22 ± 0.07	3.63 ± 0.15	4.23 ± 0.22	4.28 ± 0.22	4.93 ± 0.64	4.82 ± 0.22	§
Cl ⁻	115.95 ± 3.67	112.40 ± 1.22	114.83 ± 2.03	108.67 ± 2.68	113.07 ± 1.67	113.80 ± 2.30	
Ca ²⁺	1.05 ± 0.04	1.18 ± 0.04	1.06 ± 0.07	4.60 ± 3.60	1.02 ± 0.01	0.99 ± 0.04	

pH=arterial pH, Lactate=plasma lactate concentration (mmol L⁻¹), SvO₂=venous oxygen saturation (%), Glu=serum glucose concentration (mg dL⁻¹), Na⁺ (mmol L⁻¹), K⁺ (mmol L⁻¹), Cl⁻ (mmol L⁻¹), Ca²⁺ (mmol L⁻¹). Data are expressed as mean ± SEM.

§ =p<0.05 following RM ANOVA.

Table 6

Cytokines

	Source	BL (n = 5*)	T6 (n=5*)	T12 (n=5*)	T24 (n=3*)	T36 (n=2*)	T48 (n=2*)	p>0.05
TNF- α	Plasma (pg/mL)	92.1 $\pm$ 14.1	235.1 $\pm$ 48.1	191.7 $\pm$ 34.9	239.0 $\pm$ 87.0	631.2 $\pm$ 337.3	852.8 $\pm$ 79.1	§
	PFluid (pg/ng)	0.0092 $\pm$ 0.0073	N/A	0.364 $\pm$ 0.135	0.741 $\pm$ 0.341	0.861 $\pm$ 0.051	0.837 $\pm$ 0.010	§
	BALF (pg/ng)						0.104 $\pm$ 0.103	
IL-8	PFluid (pg/ng)	0.00278 $\pm$ 0.002	N/A	1.513 $\pm$ 0.374	3.593 $\pm$ 0.937	4.761 $\pm$ 0.892	4.749 $\pm$ 1.381	§
	BALF (pg/ng)						0.255 $\pm$ 0.068	
IL-6	Plasma (pg/mL)	39.1 $\pm$ 0.0	4814.6 $\pm$ 1715.4	4404.4 $\pm$ 22445.0	1401.1 $\pm$ 789.6	23702.0 $\pm$ 21565.8	8728.0 $\pm$ 3394.7	
	PFluid (pg/ng)	0.0125 $\pm$ 0.003	N/A	1.790 $\pm$ 0.421	1.669 $\pm$ 0.441	1.073 $\pm$ 0.226	1.488 $\pm$ 0.302	§
	BALF (pg/ng)						0.539 $\pm$ 0.094	
IL-1 β	Plasma (pg/mL)	230.8 $\pm$ 0.0	120.8 $\pm$ 49.6	146.2 $\pm$ 32.2	157.4 $\pm$ 39.2	389.3 $\pm$ 151.5	424.6 $\pm$ 60.3	§
	PFluid (pg/ng)	0.0355 $\pm$ 0.0332	N/A	0.433 $\pm$ 0.078	1.260 $\pm$ 0.301	2.580 $\pm$ 0.341	1.121 $\pm$ 0.142	§
	BALF (pg/ng)						0.493 $\pm$ 0.130	
IL-12	Plasma (pg/mL)	540.0 $\pm$ 93.4	319.4 $\pm$ 42.4	190.3 $\pm$ 43.0	176.0 $\pm$ 21.5	380.6 $\pm$ 74.2	403.1 $\pm$ 93.9	§
	PFluid (pg/ng)	0.00954 $\pm$ 0.0038	N/A	0.0656 $\pm$ 0.0249	0.144 $\pm$ 0.022	0.133 $\pm$ 0.065	0.133 $\pm$ 0.021	§
	BALF (pg/ng)						0.000947 $\pm$ 0.000021	
IL-10	Plasma (pg/mL)	31.5 $\pm$ 0.0	163.1 $\pm$ 102.2	32.0 $\pm$ 4.0	113.7 $\pm$ 56.8	40.4 $\pm$ 2.9	77.2 $\pm$ 0.0	
	PFluid (pg/ng)	0.00032 $\pm$ 0.000086	N/A	0.0155 $\pm$ 0.0082	0.0387 $\pm$ 0.0051	0.0124 $\pm$ 0.0123	0.0239 $\pm$ 0.008	
	BALF (pg/ng)						0.00094 $\pm$ 0.00002	
TGF- β	Plasma (pg/mL)	2815.1 $\pm$ 346.4	3138.9 $\pm$ 416.3	2315.5 $\pm$ 573.2	2822.1 $\pm$ 813.6	2199.5 $\pm$ 941.5	1935.7 $\pm$ 758.7	
	PFluid (pg/ng)	0.0323 $\pm$ 0.0122	N/A	0.0737 $\pm$ 0.0265	0.110 $\pm$ 0.0495	0.132 $\pm$ 0.0345	0.167 $\pm$ 0.0889	
	BALF (pg/ng)						0.5575 $\pm$ 0.2843	

TNF- α =tumor necrosis factor, IL=interleukin-8,-6,-1 β , -12,-10, TGF- β =transforming growth factor beta. Data are expressed as mean $\pm$ SEM.

§ =p<0.05 following RM ANOVA

Table 7

Ideal Animal Model of Septic Shock

Necessary components of the ideal animal model	Our model
Chronic nature of injury	Yes
Ability for continuous monitoring of physiologic parameters	Yes
Ability for repetitive blood sampling	Yes
Similar anatomy and physiology to humans	Yes
Similar immune response	Yes
Supportive treatment similar to humans	Yes
Technically easy to perform and reproducible	Yes
Polymicrobial infection and bacteremia	Yes
Varied genetic background	Yes
Varied age of animals	No
Inclusion of both sexes	No
Inclusion of comorbidities	No