UNCLASSIFIED

Optimization of a Porcine Sepsis Model with Antibiotic-Resistant Bacteria and Extremity Injury

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Introduction

Extremity trauma constitutes most of the injuries sustained on the battlefield. Delaying surgical care for these injuries will result in wound infections that may progress into life-threatening sepsis. Current criteria for sepsis diagnosis are based on combinations of patient vitals and time-consuming laboratory values. The increase in infection and subsequent sepsis is anticipated to increase in Large Scale Combat Operations when prolonged casualty care (PCC) is expected.

Objective/Hypothesis

Objective: To evaluate new approaches for the management of sepsis patients in a PCC scenario, a reproducible animal model that mimics the care and conditions of that environment is required. We are optimizing a porcine sepsis model by both topically and systemically administering multidrug-resistant pathogens to our existing complex extremity trauma injury (CETI) model.

Hypothesis: We hypothesize that the Gram-Positive (G+) and Gram-Negative (G-) Multi-Drug Resistant (MDR) pathogen inoculation parameters can be determined to administer to each animal that will result in a sustained septic state based on meeting both scoring system criteria and either pathogen confirmation in the blood or organs.

Methods

Swine (Sinclair pig) were provided appropriate anesthesia and analgesia for all procedures. Prior to CETI, animals were instrumented with a wireless telemetry device to record heart rate, blood pressure, respiration rate, and temperature and with a carotid catheter to collect blood for laboratory values throughout the study period. The CETI consisted of a muscle excision, boney defect, and thermal injury in the lower hindlimb, a hemorrhage (30-40% estimated blood volume), an extended shock period, and a 2-hour tourniquet application. A combination of MDR G+ and G- bacteria commonly associated with hospital acquired infections were inoculated topically and by intraosseous (IO) infusion at the injury site. After injury, conscious animals were assessed a minimum of twice daily. This included checking for pain and neurological responses and recording vitals. Blood was collected for microbiological testing, complete blood cell counts, and analysis of standard biochemical parameters. Approximately 4 days after injury, or when criteria was met, the animals were sedated and euthanized. Blood and tissues were collected for quantitative microbiology.

Methods

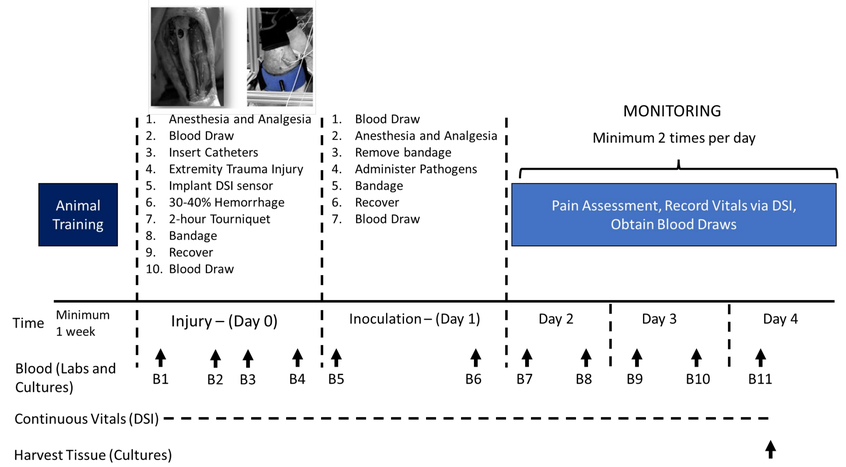

Figure 1: Timeline of experimental procedures to refine parameters for the development of the CETI Sepsis Model. Animals received at least 1 week of animal behavior training to encourage animal comfort toward research team prior to any surgery. Injury day included the catheters, wireless telemetry (DSI), and the CETI injury. Pathogens were administered on the following day. Post injury monitoring and blood was collected at designated timepoints until euthanasia.

Results

Pig #	CFU G + (volume ml)	CFU G – (volume ml)	ST Post Infection	SIRS			SOFA			NEWS2		
				Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1	4×10^9 (202)	6×10^9 (250)	67 hrs	✓	✓	✓	✗	✗	✗	✓	✓	✓
2	4×10^9 (238)	8×10^8 (210)	24 hrs	✓	N/A	N/A	✓	N/A	N/A	✓	N/A	N/A
3	4×10^9 (250)	7×10^8 (33)	16 hrs	✓	N/A	N/A	✓	N/A	N/A	✓	N/A	N/A
4	1×10^9 (250)	1×10^9 (228)	48 hrs	✓	✓	N/A	✓	✗	N/A	✓	✓	N/A

Figure 2: Summary of colony forming units (CFU) administered in saline, Survival Time (ST) post infection, and whether criteria were met for 3 different scoring systems: SIRS (systemic inflammatory response syndrome), SOFA (sequential organ failure assessment), and NEWS2 (national early warning system 2) are 3 scoring systems recommended in the CPGs. We have determined the criteria ranges for the Sinclair pigs and compared daily values to determine if they met the criteria. **X** indicates did not meet, **√** indicates does meet, N/A is because animal euthanized prior to time point. G+ = *Staphylococcus aureus*; G- = *Acinetobacter baumanii*.

Results

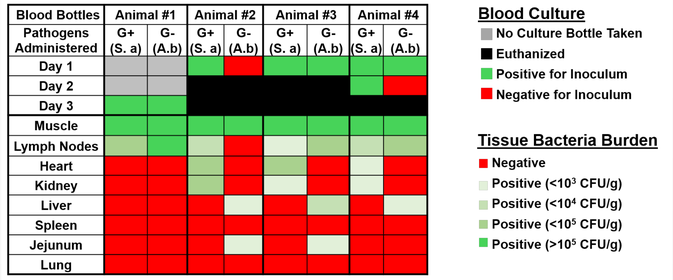

Figure 3: Heatmap summarizing the bacterial burden recovered from blood cultures collected throughout the study and from tissue homogenates at time of euthanasia. All animals have been confirmed to have a clinical infection of both pathogens in the injured muscle (*Peroneus tertius*) at time of euthanasia. All four animals tested positive for the G+ in all blood bottles throughout the study. Animal #4 cleared the G– by the time they were euthanized. Pathogens were recovered in at least 1 organ from all 4 animals.

Conclusion

In this model, we were able to induce bacteremia and septicemia following a complex extremity injury in swine. Animals met a number of necessary criteria meant to indicate a septic state. Interestingly, bacterial dissemination pattern may be materializing; G+ in heart and kidney while G– may prefer liver and jejunum. Ultimately, this will aid in the testing of therapeutics and diagnostics for identifying and treating sepsis, especially relevant to the PCC environment.

References:
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Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AAALAC International.

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