A New Survivable Damage Control Model Including Hypothermia, Hemodilution, and Liver Injury

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Background. The purpose of this article is to describe a new model of traumatic intra-cavitary hemorrhage in a hypothermic, hemodiluted liver injury model that incorporates damage control principles and allows for survival.

Materials and Methods. Twenty swine underwent a standardized 35% blood volume hemorrhage followed by resuscitation. Ten animals sustained nonsurgical and 10 a surgical high-grade liver injury. In the surgical liver injury, damage control gauze packing was performed. No operative treatment was provided for the nonsurgical liver injury, which was designed to test the efficacy of systemic hemostatic agents. After a 15min treatment phase, the abdominal cavity was closed, with the packing in place for the surgical injury, and all animals were resuscitated. Necropsy was performed at 48h post-injury.

Results. At the time of liver injury, the animals were hemodiluted and hypothermic. Both injuries caused a 20% drop in the mean arterial pressure from baseline ($P < 0.001$). Comparing baseline thromboelastography results with the results after hemodilution, hypothermia, and liver injury, a hypercoagulopathic state was observed. Mortality was 30% for both types of liver injury. The mean volume of intra-abdominal blood present at autopsy was similar for both types of liver injuries (202 ± 161mL and 214 ± 203mL, respectively).

Conclusion. A new model of traumatic intra-cavitary hemorrhage in a hypothermic, hemodiluted liver injury model with damage control that allows for survival has been described. The mortality rate of 30% allows for the comparison of therapeutic interventions that may lead to improved survival. Published by Elsevier Inc.

Key Words: damage control; animal model; liver injury; trauma; hypothermia; hemodilution; coagulopathy.

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INTRODUCTION

Hemorrhage following trauma remains a major cause of preventable mortality among adults [1–3]. Intra-cavitary injury is a primary source of traumatic bleeding. Despite advances in trauma care, an American Association for the Surgery of Trauma Organ Injury Scale (AAST-OIS) grade V liver injury, for example, still results in greater than 50% mortality [4, 5]. Compounding this, trauma patients are highly susceptible to the development of coagulopathy with a large proportion of severely injured patients arriving coagulopathic at hospital admission [6–10]. This coagulopathy contributes significantly to further hemorrhagic blood loss and is a result of several mechanisms. These include hemodilution, consumption of coagulation factors, hyperfibrinolysis, and hypothermia-induced platelet dysfunction [7, 11–14]. It is critical to be able to model this highly lethal injury scenario of intra-abdominal bleeding requiring damage control surgery in order to explore potential preventative and therapeutic strategies. To examine the short- and long-term outcome of these treatment strategies, damage control with survival is a prerequisite. To date there is no survivable damage control trauma model of intra-abdominal hemorrhage described in the literature [15]. Several models of traumatic liver injuries exist in swine [16–25],

however, these models were not designed to incorporate principles of damage control.

The purpose of this article is to describe a new model of traumatic intra-cavitary hemorrhage in a hypothermic, hemodiluted liver injury model with damage control that allows survival.

MATERIALS AND METHODS

All study procedures were carried out between November 2007 and August 2008. The study was conducted at the University of Southern California (USC) Health Science Research facility, which is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International and has an animal welfare assurance number (A3518-01) on file with the NIH Office of Laboratory Animal Welfare. The Institutional Animal Care and Use Committee approved the study design, and all animal subjects were cared for in strict compliance with the National Institutes of Health Guide for the care and use of Laboratory Animals (86-23, revised 1996).

Experimental Animals

Twenty female Yorkshire-Hampshire swine approximately 3 mo old with a mean weight of 39.8 ± 2.7 kg were obtained from IFPS Inc. (Norco, CA). All animals were fed a standard diet and were housed in quarantine for 7 d preoperatively. Food was withheld the night before the procedure with free access to water.

Anesthesia

Each animal received premedication consisting of an intramuscular injection of 4 mg/kg of tiletamine/zolazepam and xylazine, followed by 0.01 mg/kg of glycopyrrolate. Peripheral intravenous access was established using a 20 G catheter placed in the marginal ear vein. All animals were intubated using a 7 French endotracheal tube and maintained on a Narkomed 4 anesthesia system with sevoflurane 1%–5%. Core body temperature was monitored *via* an esophageal thermistor probe, and a gastric tube was inserted to decompress the stomach.

The right carotid artery was cannulated using a cutdown technique with a 20 G angiocatheter inserted for arterial blood pressure monitoring and blood draws. The right external jugular vein was cannulated with a 9 Fr introducer sheath to be used for blood draws and administration of resuscitative fluids.

Hemodilution and Hypothermia

Each animal underwent a standardized 35% blood volume (24.5 mL/kg, based on a 7% of body weight blood volume) hemorrhage through the external jugular vein sheath over a 15–30 min period. The blood draw was followed by a resuscitation period during which 1000 mL of room temperature (19–21 °C) lactated Ringer's solution (LRS) was given to maintain an invasive mean arterial pressure (iMAP) of 60 mmHg or greater, and to lower the body temperature.

After the initial hemodilution phase, a midline laparotomy was performed. The body temperature was further lowered by the intra-abdominal placement of two frozen 500 mL LRS bags, wrapped in sterile towels to achieve a core temperature of 35 °C. Subsequently, in 10 animals, a nonsurgical liver injury, and in 10 animals, a surgical liver injury (AAST-OIS grade IV) [26] was created.

Liver Injury and Damage Control Surgery

To create the surgical liver injury, a scalpel blade was used to make a 0.5 cm deep longitudinal incision 10 cm in length beginning 5 cm from the inferior edge and 3 cm from the medial edge of the left middle lobe, with right angle extensions at its superior and inferior ends to create a 3×10 cm rectangular segment. This 3×10 cm segment was subsequently avulsed off the lobe using a crushing clamp, injuring the left middle hepatic vein and exposing a large area of liver parenchyma (Fig. 1A and B). After 2 min of uncontrolled bleeding, damage control liver packing with 20 4×4 cm gauze pads was performed. This bleed is utilized as an internal control to ensure uniformity of the injury prior to any active treatment. After the 15 min treatment phase, the gauze was removed to quantify the shed blood, and the liver was repacked with 20 4×4 cm gauze pads and the abdomen closed with the liver packing in place.

The nonsurgical liver injury was created by making two 5 cm $\times 5$ cm grids with lacerations 1 cm apart and 0.5 cm in depth. These were created on the diaphragmatic surface of the left and left middle lobes of the liver (Fig. 2). No packing was utilized, and shed blood was measured at 2 and 15 min. In both types of liver injuries, shed blood was collected with pre-weighed sponges at 2 and 15 min post-injury and weighed.

Resuscitation and Conclusion of the Experiment

At the conclusion of the experiment, the animals were resuscitated with warm LRS to maintain an iMAP > 60 mm Hg and increase body temperature to 37 °C. They were then extubated. Maximal allowed intravenous volume was 3500 mL of LRS per animal. During the operation, surgical facial edges were infiltrated with 0.5% bupivacaine, and postoperatively the animals received scheduled analgesic medication. During the entire 48 h follow-up, the animals were closely observed with repeated clinical examinations by a veterinarian. At 48 h post-injury, a tiletamine/zolazepam and xylazine premedication dose of 2.2 mg/kg was given intramuscularly, followed by euthanasia of the animal using sodium pentobarbital 120 mg/kg IV overdose immediately prior to necropsy.

Blood Work

Arterial and venous blood samples were analyzed with the hand held iStat 1 Analyzer (Abbott Laboratories, Abbott Park, IL.) and the Ac-T Hematology Analyzer (Beckman Coulter, Inc., Fullerton, CA). Both analyzers utilize whole blood, and were performed at the bedside. Blood assays included hemoglobin, hematocrit, platelet count, pH, lactate level, base deficit, anion gap, partial prothrombin time (PTT), prothrombin time (PT), international normalized ratio (INR), and were measured at three time points: baseline, after blood withdrawal and resuscitation period, and after the liver injury.

Thromboelastography (TEG) (TEG 5000 Thromboelastograph Hemostasis Analyzer; Haemoscope Corporation, Niles, IL) was performed at baseline and 20 min after the liver injury. TEG provides a complete clotting profile analysis, including time until the first evidence of a clot (R time), speed of clot formation (K time or angle α), clot strength (maximum amplitude: MA), and fibrinolysis. This assay was performed at bedside using 360 μL of fresh whole blood.

Data Points Extracted and Statistical Analysis

The data points accrued were iMAP, heart rate (HR, bpm), body temperature (°C), volume of the initial blood withdrawal (mL), volume of LRS during the resuscitation period (mL), volume of shed intra-abdominal blood 2 and 15 min after the liver injury, and intra-abdominal blood volume immediately after sacrifice 48 h post-injury.

The values are expressed as mean ± SD or ± SEM. Repeated-measures ANOVA were used to analyze the effects of the initial blood withdrawal and fluid resuscitation on the invasive mean arterial pressure (iMAP, mmHg), heart rate (HR, beats per min [bpm]), and body temperature (°C). Dependent and independent continuous variables were compared using Wilcoxon and Mann-Whitney, respectively. Categorical data were compared using χ^2 test. A P