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TITLE: The Effect of Hypotensive Resuscitation and Fluid Type on Mortality, Bleeding, Coagulation, & Dysfunctional Inflammation in a Swine Grade V Liver Injury Model

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14. ABSTRACT Objectives: To determine the optimal fluid resuscitation and anesthetic regimen for swine undergoing uncontrolled hemorrhage. To develop a severe multi-system trauma model resulting in the lethal triad. Methods: 1. 20 swine underwent Grade V liver injury followed by 30 minutes of hemorrhagic shock without resuscitation. Hemodynamics were measured using PICCO 2. Systemic and local lung inflammation was measured in animals undergoing TIVA and isoflurane anesthetic. 3. Femur fracture, controlled hemorrhage, hypothermia and liver injury were combined to create a reproducible model replicating the lethal triad. Results: 1. Resuscitation with NS results in decreased SVR and increased CO as well as increased extravascular lung water. This suggests that NS is more likely to predispose trauma patients to ARDS. 2. A systemic pro-inflammatory response can be measured within 2 hours of injury and shock. Anesthesia with TIVA produces suppression of TNF-alpha mRNA in the lung compared to anesthesia with isoflurane. 3. A severe reproducible multi-system injury can be created in swine with good short term survivability. This injury model can be reliably reproduced at multiple distant centers.					
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INTRODUCTION:

Exsanguination is the leading cause of death on the battlefield. Lifesaving interventions include arresting hemorrhage and initiating resuscitation. The ideal resuscitation of combat casualties has not been determined. Delaying resuscitation has been shown to be beneficial in some settings and anesthetics utilized can have a profound effect on the resuscitation. The goal of this proposal is to determine the ideal resuscitation regimen of swine undergoing a Grade V liver injury followed by 30 minutes of uncontrolled hemorrhagic shock. Fluids studied include lactated Ringer's (LR), normal saline (NS). The hemodynamic effects of the fluids were studied utilizing pulse contour wave analysis (PiCCO). In addition to fluid resuscitation, anesthetics play a critical role in the outcome of trauma victims. The ideal anesthetic would be easy to administer, have minimal hemodynamic effects and minimal long term effects. Utilizing blood and tissues from previously studied animals, the effect of total intravenous anesthesia (TIVA) on inflammatory mediators was also studied.

There is increasing evidence that aggressive resuscitation with blood components will improve survival in severely injured trauma victims by correcting coagulopathy early and reducing blood loss. This has been termed hemostatic resuscitation. Our group was asked to develop a severe multi-trauma model that recreates the lethal triad of hypothermia, coagulopathy and acidosis. This model will be used by 3 centers to study the effects of hemostatic resuscitation.

BODY:

Materials and Methods

Part 1 – Hemodynamic Effects of lactated Ringer's and normal saline.

This was a randomized controlled trial using twenty female Yorkshire crossbred pigs. The pigs underwent a 16-hour pre-operative fast except for water ad libitum and were pre-anesthetized with an intramuscular injection of 8 mg/kg Telazol[®] (Fort Dodge Animal Health, Fort Dodge, Iowa). They then underwent oral tracheal intubation with a 7.0 mm or 7.5 mm endotracheal tube and were placed on mechanical ventilation. Respiratory rate was adjusted to keep pCO₂ values between 40-50 torr. Anesthesia was maintained using 2% isoflurane in 100% oxygen. An esophageal thermometer was inserted.

Animal temperature was controlled utilizing external warming devices. Once the swine were anesthetized, left cervical cut downs were performed and a central venous polyethylene catheter was inserted into the external jugular vein. The venous line was used for administration of the resuscitation fluids. Femoral artery cut down was performed to place a 4-F aortic catheter with an integrated thermistor tip (Pulsion Medical Systems, Munich, Germany) for continuous blood pressure monitoring and blood sampling. Mean arterial pressure (MAP) and heart rate (HR) were continuously recorded using PiCCO-Technology that was connected to the PiCCO *plus* monitor (Pulsion Medical System, Munich, Germany).

The PiCCO technology system allows hemodynamic monitoring through two different techniques, either intermittently by transpulmonary thermodilution or continuously by pulse contour wave analysis. It is a validated, less invasive alternative to the Swan-Ganz catheter for

the measurement of cardiac output (CO). For transpulmonary thermodilution, a bolus (15 ml per bolus) of cooled (0-6°C) crystalloid fluid was injected through a venous catheter and the thermistor tipped arterial catheter placed through the femoral artery would measure the subsequent temperature changes. These measurements were done manually and randomly throughout the respiratory cycle to obtain CO and SV measurements and calculated systemic vascular resistance values. Thermodilution is also used for calibration of the pulse contour method for continuous measurements of stroke volume (SV) and CO. Using this technology, several other parameters can be measured to include extravascular lung water and pulse pressure variation. Increases in extravascular lung water have been correlated with the development of ARDS.

The animals underwent a midline celiotomy, suprapubic Foley catheter placement, and splenectomy. Splenectomies are performed in swine hemorrhage models because of the spleen's distensibility and the resultant variation in amounts of sequestered blood. The spleen was weighed and, based on randomization, either LR or NS was infused to replace three times the spleen weight. Cystostomy was performed and a foley catheter was placed to measure urine output. The abdomen was then closed with towel clamps.

Following a 15-minute stabilization period, the abdomen was opened and residual peritoneal fluid was removed. Pre-weighed laparotomy pads were placed in both paracolic gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury (injury to a central hepatic vein) was created with a specially designed clamp. The clamp was positioned in the middle of the liver, placing the right hepatic vein, the left hepatic vein, and the portal vein at risk for injury. This technique resulted in injuries consistent with grade V injuries defined by the American Association for the Surgery of Trauma Organ Injury Scaling System. This model has been described in several prior studies. The time of injury was considered the start time of the two-hour study period. Following 30 minutes of uncontrolled hemorrhage, the initial blood loss, measured by wall suction and the pre-weighed laparotomy pads, was determined. The abdomen was then closed.

We blindly randomized (using a random numbers table) the swine to receive either NS or LR resuscitation at 165 ml/min. This rate is approximately one half the rate delivered by the Level 1 rapid infuser[®] as the animals were approximately one half the weight of an average human. Resuscitation fluid was administered to achieve and maintain the baseline MAP for 90 minutes post-injury.

Upon completion of the 2-hour study period, the abdomen was reopened and the secondary blood loss was determined by adding the volume of intra-abdominal blood to the weight of the intra-abdominal blood clots. Following the completion of the study the animals were sacrificed by exsanguination. To ensure comparable injuries between the study groups, we removed the liver and identified the number of hepatic vessels injured.

Part 2 – Comparison of the effects of Isoflurane anesthesia and TIVA anesthesia on systemic inflammation and local mRNA production in the lung.

This was a randomized controlled trial using twenty-six female Yorkshire crossbred swine. The animals were fasted for 16 hours prior to surgery, except for water ad libitum. We pre-anesthetized the swine with an intramuscular injection of 8mg/kg Telazol[®] (Fort Dodge Animal Health, Fort Dodge, Iowa), followed by induction with 2% ISO. Orotracheal intubation

was performed with a 7.0mm or 7.5mm internal diameter cuffed endotracheal tube, and the animals were placed on mechanical ventilation. Respiratory rate and tidal volume were adjusted to keep pCO₂ values between 40-50 torr. An esophageal thermometer was placed, and the animal temperature was maintained at $38.0 \pm 1.5^{\circ}\text{C}$ using external warming devices.

Six swine were randomized to a control arm and underwent sacrifice and tissue harvesting after induction of anesthesia. Cytokine mRNA levels from these animals served as baseline data for the population.

Following induction, an 18 gauge aural intravenous (IV) catheter was placed. Animals then were switched to the blindly randomized (using a random numbers table) maintenance anesthesia consisting of either 1-3% ISO, or a TIVA regimen consisting of IV ketamine (15-33mg/kg/hr), midazolam (1-2mg/kg/hr), and buprenorphine (0.5-1 mcg/kg/hr). These doses fall within the normal, therapeutic range for swine. The ISO group received an equivalent volume of lactated Ringer's solution (LR) instead of the IV medications to standardize the volume of fluid administered. The level of sedation was constantly monitored by an animal technician independent of the study team through measurement of jaw laxity, hemodynamic fluxuations, and response to painful stimuli at the nasal septum and forefoot. All efforts were made to ensure the study team remained blinded to the anesthetic regimen.

A left ventral cervical cut down was performed and 8F polyethylene catheters were inserted into the common carotid artery, external jugular vein, and internal jugular vein. The arterial catheter was used for continuous blood pressure analysis and blood sampling. Mean arterial pressure (MAP), and heart rate (HR) were continuously recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer (DigiMed, Louisville, Kentucky). The external jugular catheter was used for fluid resuscitation. The infusion of either the TIVA medications or LR (in the ISO group) was switched from the aural catheter to the internal jugular vein catheter upon its placement.

The animals underwent a midline celiotomy, suprapubic Foley catheter placement, and splenectomy. Splenectomies are performed in swine hemorrhage studies because the swine spleen is distensible and contains highly variable amounts of blood that can act as an auto-transfusion. The spleen was weighed and LR was infused to replace three times the spleen weight in grams. The abdomen was then closed with towel clamps.

Following a 15-minute stabilization period, the blood pressure was recorded and used as the baseline MAP. The abdomen was opened and residual peritoneal fluid was removed. Pre-weighed laparotomy pads were placed in both paracolic gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury (injury to a central hepatic vein) was created using a specially designed clamp. The clamp was positioned in the middle of the liver, placing the right hepatic vein, left hepatic vein, and portal vein at risk for injury. This protocol is based upon our previous studies of uncontrolled hemorrhagic shock using this same model.³² The time of injury was considered the start of the study (time point 0). During hemorrhage, the anesthetic regimen was stopped when the MAP was below 30 mmHg, and restarted upon rising above 30mmHg for both groups. Following 30 minutes of uncontrolled hemorrhage, the initial blood loss was determined using wall suction and the pre-weighed laparotomy pads. The abdomen was then closed. A fixed volume of LR was administered at 8ml per ml of measured blood loss at 165ml/min. This volume of fluid was calculated based upon our previous studies on the amount of LR required to maintain the baseline blood pressure for 2 hours following liver injury. The

rate of administration is approximately one half the rate delivered by the Level 1 rapid infuser[®] as the animals were approximately one half the weight of an average human.

Upon completion of the 2-hour study period, the abdomen was reopened and the secondary blood loss was determined by adding the volume of intra-abdominal blood and the weight of the intra-abdominal blood clots. Following completion of the study the animals were sacrificed and lung tissues harvested. To ensure comparable injuries between study groups, we removed the liver post-mortem and analyzed the number of hepatic vessels injured.

Blood specimens were collected at baseline and every 30 minutes until completion of the 2-hour study. Blood assays included lactate, arterial blood gas, chemistry panel, liver function tests, and hematocrit. Serum for cytokine analysis was collected at baseline and at study completion. Lung tissues harvested were immediately placed in RNeasy[™] solution (Ambion, Austin, Texas) and stored at -80°C.

=Serum and Tissue Cytokine Analysis

Serum cytokine levels were quantified using the Quantikine[®] enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). Lung tissue levels of IL-6, IL-8, and TNF- α mRNA were determined using the technique of quantitative, real-time reverse transcriptase polymerase chain reaction (RT-PCR). β -actin was used as an endogenous control. Total RNA was isolated from RNeasy[™]-stored lung tissue using a commercially available kit (RNeasy[®] Mini Kit; Qiagen, Valencia, CA). The extracted RNA concentration was determined with a spectrophotometer based on the absorbance at 260nm. Two micrograms of RNA was reverse-transcribed into cDNA using the SuperScript[™] III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) using random hexamers, according to the package protocol. Twenty-five nanograms of cDNA was used for performing quantitative RT-PCR using the Applied Biosystems 7900HT (Applied Biosystems; Foster City, CA) under the following conditions: Stage 1) 2 minutes at 50°C, Stage 2) 10 minutes at 95°C, Stage 3) 40 cycles of 15 seconds of melting at 95°C followed by DNA synthesis for 1 minute at 60°C.

Primers and probes used for specific PCR amplification and quantification of swine β -actin, IL-6, IL-8, and TNF- α mRNA were derived from published swine sequences.³³⁻³⁶ Primers and probes were created (using the Assays-by-Design software from Applied Biosystems) to bind at unique, and individualized sites on the gene of interest to reduce interference. Primers and probes were used at concentrations of 18 μ M, and 5 μ M, respectively. The primer and probe sequences are as follows, each from 5' to 3':

β -actin forward primer: TCTTCCAGCCCTCCTTCCT, β -actin reverse primer:

TCGCACTTCATGATGGAGTTGA, β -actin probe: [FAM]-CCTGCGGCATCCAC-[NFQ];

IL-6 forward primer: TGCTTCCAATCTGGGTTCAATCAG, IL-6 reverse primer:

GCTCTCATACTCTTTCTGGAGGTAGT, IL-6 probe: [FAM]-TCACCACCGGTCTTGTG-[NFQ]

IL-8 forward primer: CTGGCAAGAGTAAGTGCAGAACT, IL-8 reverse primer:

GTCCACTCTCAATCACTCTCAGTT, IL-8 probe: [FAM]-CCAGTGCATAAATACG-[NFQ]

TNF- α forward primer: CAGATCATCGTCTCAAACCTCAGAT, TNF- α reverse primer:

TCCCTCGGCTTTGACATTGG, TNF- α probe: [FAM]-CCGTCGCCCACGTTGT-[NFQ]

(NFQ = Non-Fluorescent Quencher)

Statistical Analysis

An independent samples *t* test was used to compare the means of continuous variables between the two groups. Fisher's exact test was utilized when the *n* for a given data set was less than 5. Statistical significance was defined as a *p* value <0.05. Values within a group and comparisons of three or more groups were compared using a posthoc analysis of the variance (ANOVA). These values were calculated using SPSS version 13.0 software (SPSS Inc., Chicago, IL). Graphs were produced using Microsoft Excel 2003 (Microsoft Inc., Redmond, WA), and Origin[®] 6.0 (Microcal Software Inc., Northampton, MA).

Part 3 – Development of a severe multi-system trauma model that replicates the lethal triad.

Yorkshire swine were anesthetized with isoflurane, intubated and instrumented for monitoring. The anesthesia and line placement has been previously described. A femur fracture and soft tissue injury was then created in the area of the left groin utilizing a captive bolt gun. Following this injury, animals underwent a 60% controlled total blood volume hemorrhage. Mean arterial pressure was monitored continuously throughout this period and if the blood pressure dropped to less than 25 mmHg, hemorrhage was stopped and animal were resuscitated with NS to a blood pressure of 30 mmHg. Animals underwent a celiotomy and placement of a suprapubic catheter. Utilizing cooled fluids their temperature was lowered to 33C. Following controlled hemorrhage there was a 30 minute shock period. The hemorrhage volume was then replaced with normal saline given in a 3:1 ratio. Hemodynamic parameters were measured continuously. Thrombelastography (TEG), PTT, PT and laboratory values were collected at baseline, after the shock period and after NS replacement. Animals then underwent Grade V liver injury as has previously been described. Thirty seconds after injury the livers were packed.

Following creation of this model, at OHSU it was replicated at the Institute of Surgical Research and Harvard. Reproducibility of the model was compared between the 3 centers to determine if a multi-center study could be performed. The goal of the study would be to determine the optimal ratio of blood components necessary to correct coagulopathy in this severe injury model. In future, studies we also wish to develop an artificial whole blood that could be stored for prolonged periods of time.

RESULTS

Part 1

One animal in the NS died prior to completion of the study. Table 1 shows mean weight, baseline MAP, vessels injured, spleen replacement fluid, blood loss, urine output and resuscitation volume between the 2 groups.

Table 1. Comparison of LR and NS groups.

Parameter	Study Fluid	Mean $\pm$ Std. Error	Statistical Significance
Survived	NS LR	9 10	0.343
Weight (kg)	NS LR	33.6 $\pm$ 1.0 35.6 $\pm$ 0.9	0.165
Baseline MAP	NS LR	70.4 $\pm$ 2.7 68.6 $\pm$ 3	0.66
Veins injured	NS LR	1.8 $\pm$.25 1.5 $\pm$.22	0.382
Spleen replacement fluid (ml)	NS LR	627 $\pm$ 52 612 $\pm$ 33	0.811
EBL after injury (ml)	NS LR	763 $\pm$ 65 649 $\pm$ 50	0.182
Resuscitation volume received (ml)	NS LR	10901 $\pm$ 1208 5175 $\pm$ 622	0.001 *
Urine output (ml)	NS LR	1459 $\pm$ 280 652 $\pm$ 124	0.021 *

Injuries and blood loss were similar between groups. Animals receiving NS had twice the fluid requirement and significantly increased urine output. Despite infusion at 165ml/min, we were unable to maintain the pre-injury MAP in some of the NS pigs toward the end of the resuscitation period. Table 2 shows the pH and lactate levels at baseline and 30 minute time intervals until the end of study. Despite similar pH levels at baseline, the NS animals were found to be more acidotic from 30 minutes following injury to the end of the study compared to the LR animals ($p<0.05$).

Table 2. pH values compared throughout the study.

	Study Fluid	Baseline	T30	T60	T90	T120
pH	NS	7.49 $\pm$ 0.03	7.42 $\pm$ 0.01*	7.31 $\pm$ 0.02*	7.24 $\pm$ 0.02*	7.17 $\pm$ 0.03*

	LR	7.51±0.01	7.47±0.01	7.43±0.01	7.44±0.01	7.41±0.01
Lactate	NS	1.73±0.14	2.36±0.30	1.71±0.25*	1.34±0.29*	1.30±0.28*
(mmol/L)	LR	2.43±0.30	3.25±0.41	5.47±0.60	4.82±0.37	5.99±0.70

Figure 1 shows the average MAP of the groups. MAP in the NS group was lower during the resuscitation phase despite receiving twice the infused volume which was significant from 66-97 min following injury. The mean values for the two groups again become more similar at the final time point. This is in large part due to the death of one pig in the NS group after time point 6. The pig that died prior to the end of the experiment was included in the analysis up until the point at which it died.

Figure 1. Comparison of MAP between LR and NS groups throughout the study.

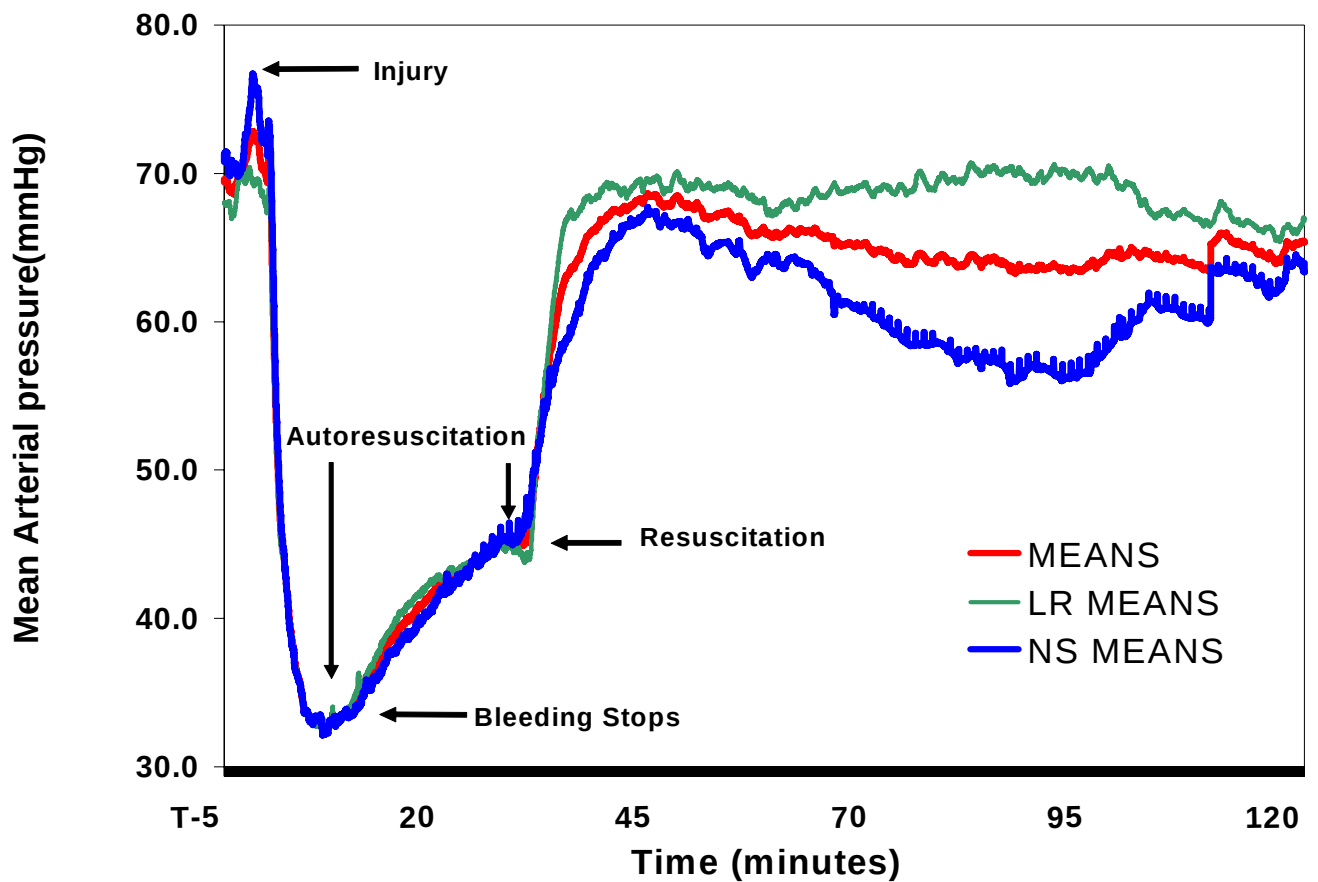
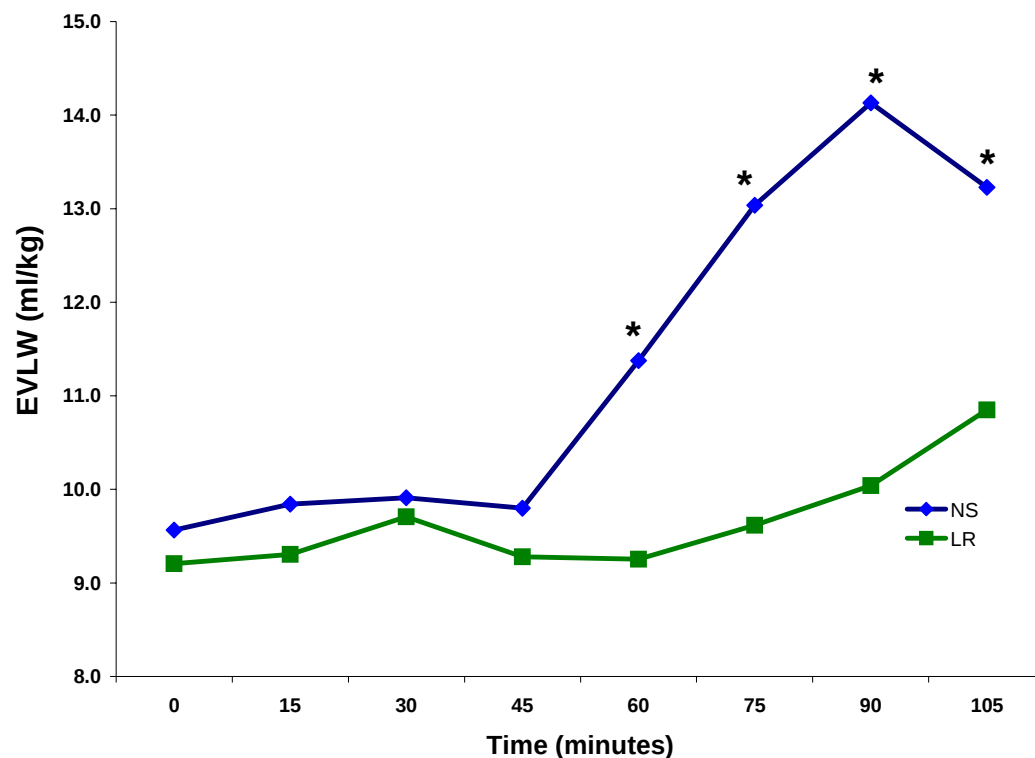


Figure 2 shows extravascular lung water compared between groups. It is notable for the fact that EVLW is immediately increased in the NS group even when resuscitation volumes are similar. This controls for the fact that NS animals received a significantly greater fluid resuscitation

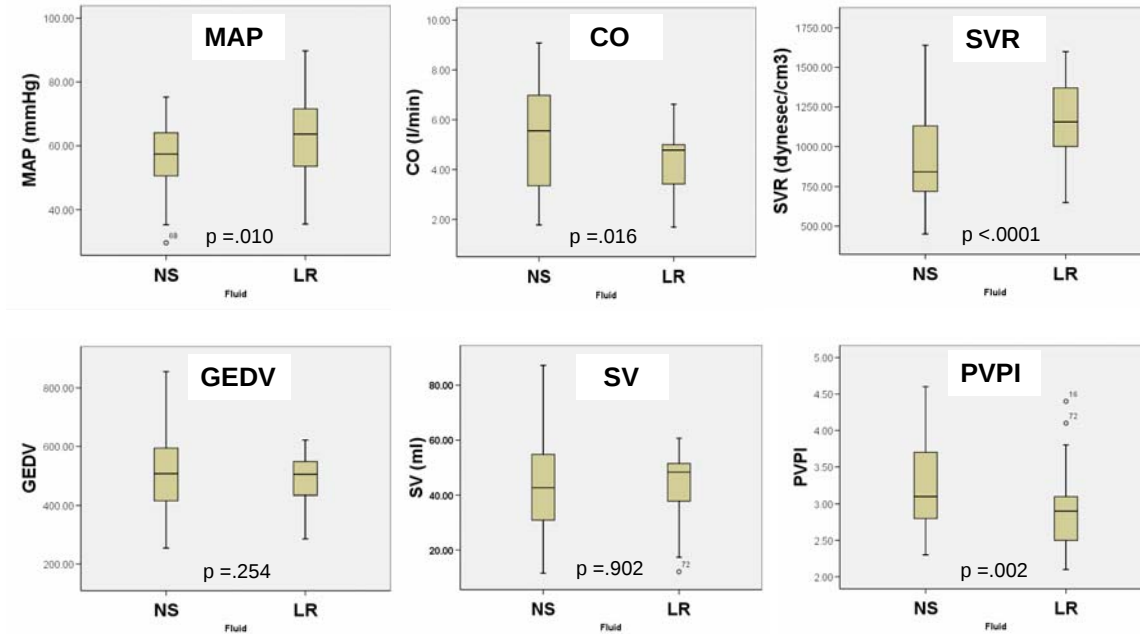
compared to LR pigs. This suggests that NS is more likely to predispose patients to the development of ARDS.

Figure 2. Extravascular lung water compared between groups.



The effect of the fluid resuscitation regimens on hemodynamic parameters is shown in Figure 5. As figure 5 reveals, NS resuscitation results in marked reduction in SVR and increase in cardiac output. Stroke volume and global end diastolic volume remain similar between the groups. We hypothesize that these hemodynamic changes are a result of acidosis in the NS group.

Figure 3. Comparison of Hemodynamic parameters



Part 2

Ten animals were randomized to each study group, and six animals were used as controls. Two animals in each study group died prior to completion of the 2 hour study period. Both study groups were similar with respect to weight, temperature, blood loss, fluid resuscitation, total urine output (UOP), and liver injury pattern. Liver function tests were within normal limits at baseline, and were similarly elevated in both groups at the end of the study ($p > 0.1$ between groups at baseline and 120 min.).

Randomized maintenance anesthesia was administered at a minimum of 60 minutes prior to liver injury, allowing equilibration of the anesthetic for each animal. Baseline MAP was 89.5mmHg for the TIVA group compared to 76.0mmHg for the ISO group ($p = 0.022$). Similarly, MAP at injury was greater in the TIVA animals than ISO animals, 86.4mmHg vs. 66.6mmHg ($p = 0.004$). Following injury there was a rapid drop in blood pressure, followed by a period of auto-resuscitation that was similar in both groups. At 30 minutes, each group received standardized fluid resuscitation resulting in a similar rise in MAP. Three animals in the TIVA group returned to their baseline blood pressure, compared with 6 in the ISO group ($p = 0.37$). Following fluid resuscitation, the MAP was maintained in TIVA animals, but persistently decreased in ISO animals. This difference became significant near the end of the study ($p < 0.05$).

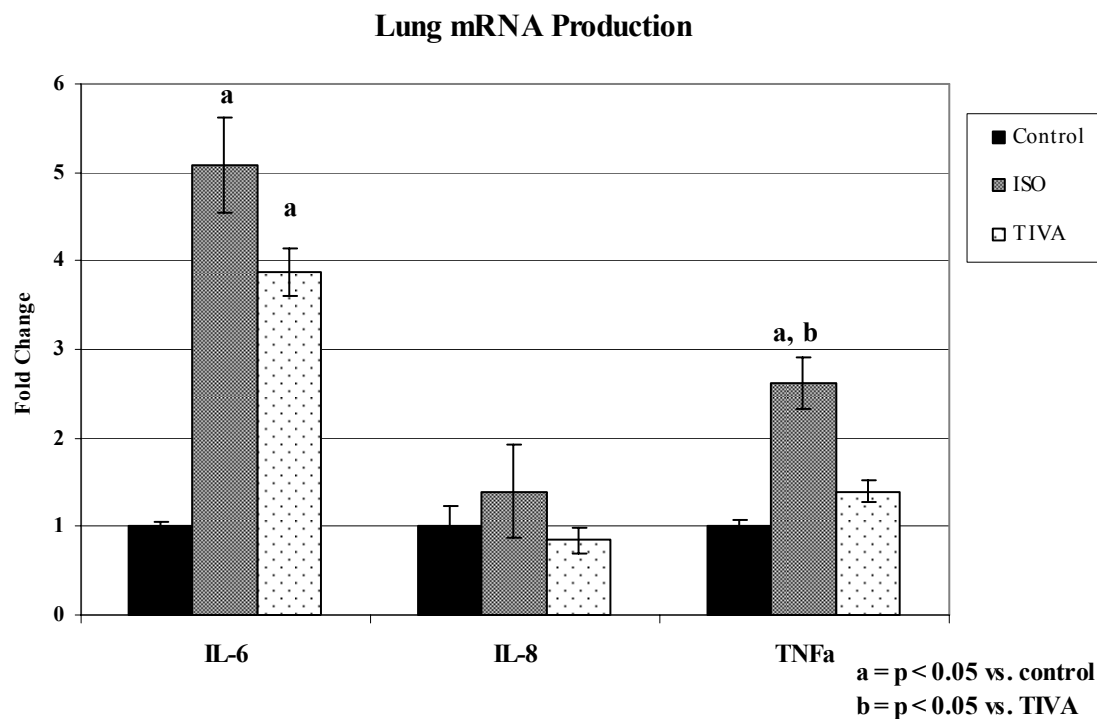
Serum cytokine levels for each anesthetic group are displayed in Table 3. Within each group there is a significant elevation of IL-6, IL-8, and TNF- α at the conclusion of the study when compared to baseline. When comparing one group to another there were no differences seen at either time point ($p > 0.1$). In comparison, Figure 4 shows mRNA production in lung tissue for TIVA, ISO, and control animals as quantified with RT-PCR. Values are represented as fold change relative to the control animals, which by definition have a value of 1. While ISO animals appear to have greater IL-6 mRNA production, there is no difference when compared to TIVA animals due to the large standard deviation. Both groups do have greater expression when compared to controls ($p < 0.001$ for TIVA and ISO vs. control). For IL-8, there are no differences between all three groups. ISO animals have elevated TNF- α mRNA production

when compared to both control and TIVA animals ($p = 0.004$, and $p = 0.043$, respectively). TIVA animals had similar TNF- α mRNA levels as controls ($p = 0.21$).

Table 3. Comparison of serum cytokines between TIVA and ISO groups.

		TIVA	ISO	p-value
IL-6 (pg/ml)	Baseline	2.2 ± 4.5	1.0 ± 1.5	0.5
	120 min.	216.8 ± 104.6	231.0 ± 162.3	0.8
	p-value	<0.001	0.005	
IL-8 (pg/ml)	Baseline	26.5 ± 20.1	43.2 ± 32.0	0.7
	120 min.	175.9 ± 74.4	311.0 ± 227.9	0.1
	p-value	<0.001	0.013	
TNF-α (pg/ml)	Baseline	52.9 ± 13.3	56.4 ± 17.1	0.2
	120 min.	118.4 ± 66.8	304.7 ± 298.5	0.1
	p-value	0.027	0.05	

Figure 4.



Part 3

Twenty-nine animals were used to complete the initial comparison of the groups. 5 animals (17%) died before completion of the study period. Mean arterial pressure after the shock period was 32 ± 2 mm Hg and was similar between centers ($p=0.4$). Mean pH, base excess, and lactate levels were 7.29 ± 0.02 , -8.20 ± 0.65 mmol/L, and 5.29 ± 0.44 mmol/L, respectively, following NS replacement. This was not different between centers ($p>0.05$). PTT, PT, and TEG R' values were different ($p<0.01$). Similar spun hematocrit levels were achieved following controlled hemorrhage ($p=0.15$) and dilution ($p=0.9$).

KEY RESEARCH ACCOMPLISHMENTS

1. Following uncontrolled hemorrhagic shock resuscitation with lactated Ringer's and normal saline result in differing hemodynamic outcomes.
2. Resuscitation with NS results in decreased SVR and increased CO.
3. Resuscitation with NS also results in increased extravascular lung water independent of the volume of fluid given.
4. Dysfunctional inflammation as measured by elevation of IL-6, IL-8 and TNF-alpha can be measured as early as 2 hours after initiation of uncontrolled hemorrhagic shock.
5. Isoflurane and TIVA anesthesia produce similar degrees of systemic inflammation at 2 hours.

6. Anesthesia with TIVA results in suppression of TNF-alpha mRNA production in the lung compared to anesthesia with TIVA.
7. A severe multi-system shock model which reliably reproduces the lethal triad can be created in swine with good survivability.
8. This shock model can be reproduced at other centers with comparable results except for coagulation parameters.

REPORTABLE OUTCOMES

Part 1 of this work was presented at the 2006 American College of Surgeons Surgical Forum session. The manuscript has been submitted to the Journal of the American College of Surgeons. An additional abstract describing extravascular lung water has been submitted to the European Shock Society in Brussels.

Part 2 of this work was presented at the 2007 meeting of the Eastern Association for the Surgery of Trauma. The presentation was the winner of the resident competition and the manuscript won the award for the best manuscript. The work was also presented at the 2006 Portland Surgical Society and the Oregon and Washington Chapter meetings of the American College of Surgeons. It was the of the best basic science paper at the Portland Surgical Society. The abstract has been published in the Journal of Trauma and the manuscript is submitted for publication in the Journal of Trauma.

Part 3 of this work has been submitted in abstract form to the Shock Society.

BIBLIOGRAPHY OF PUBLISHED WORK RESULTING FROM THIS GRANT

MANUSCRIPTS

1. Watters JM, Brundage SI, Todd SR, Zautke NA, Stefater JA, Lam JC, Muller PJ, Malinoski D, **Schreiber MA**. Resuscitation with Lactated Ringer's Does Not Increase Inflammatory Response in a Swine Model of Uncontrolled Hemorrhagic Shock. Shock. 2004; 22:283-287.
2. Brundage SI, Zautke NA, Watters JM, Stefater JA, Todd SR, Lam JC, **Schreiber MA**. Lactated Ringer's Does Not Increase Inflammation after Shock. 6th World Congress on Trauma, Shock, Inflammation and Sepsis. 2004; EC302C0210:151-155.
3. Todd SR, Malinoski DJ, Muller PJ, **Schreiber MA**. Hextend Attenuates Hypercoagulability Following Severe Injury in Swine. Journal of Trauma. 2005;59:589-594.
4. Kiraly LN, Differding JA, Enomoto TM, Sawai RS, Muller PJ, Diggs B, Tieu BH, Englehart MS, Underwood S, Wiesberg TT, **Schreiber MA**. Resuscitation with Normal Saline (NS) vs. Lactated Ringers Modulates Hypercoagulability and Leads to Increased

Blood Loss in an Uncontrolled Hemorrhagic Shock Swine Model. *Journal of Trauma*. 2006;61;57-65.

5. Watters JM, Tieu BH, Differding JA, Muller PJ, **Schreiber MA**. A Single Bolus of 3% Hypertonic Saline with 6% Dextran Provides Optimal Initial Resuscitation after Uncontrolled Hemorrhagic Shock. *Journal of Trauma*. 2006;61;75-81.
6. Watters JM, Tieu BH, Todd SR, Jackson T, Muller PJ, Malinoski DJ, **Schreiber MA**. Fluid Resuscitation Increases Inflammatory Gene Transcription Following Traumatic Injury. *Journal of Trauma*. 2006;61;300-309.
7. Todd SR, Malinoski DJ, Muller PJ, **Schreiber MA**. Lactated Ringer's is Superior to Normal Saline in the Resuscitation of Hemorrhagic Shock. *Journal of Trauma*. In Press.

ABSTRACTS

1. Todd SR, Malinoski D, **Schreiber MA**. Lactated Ringer's is Superior to Normal Saline in Uncontrolled Hemorrhagic Shock. *Shock*. 2003;Supplement to Volume 19:169.
2. Todd SR, Malinoski D, **Schreiber MA**. Hextend Attenuates the Hypercoagulability Following Severe Liver Injury in Swine. *Journal of Trauma*. 2004;56:226.
3. Stefater, Brundage SI, Watters J, Zautke N, Todd SR, Lam, **Schreiber MA**. Lactated Ringer's does not Increase Inflammation after Shock. *Shock* 2004;Supplement to Volume 21:458.
4. Differding JA, Watters JM, Muller PJ, **Schreiber MA**. The Correlation between Mean Arterial Blood Pressure and Tissue Oxygenation after Uncontrolled Hemorrhagic Shock. *Journal of Surgical Research*. 2004;126:336-337.
5. Watters JM, Jackson T, Muller PJ, Malinoski D, Todd SR, **Schreiber MA**. Fluid Resuscitation Increases Inflammatory Response to Traumatic Injury. *Journal of Trauma*. 2004;57:1378.
6. Watters JM, Differding JA, Muller PJ, **Schreiber MA**. A Single Bolus of 3% Hypertonic Saline with Dextran Provides Optimal Resuscitation after Uncontrolled Hemorrhagic Shock. *Journal of Trauma*. 2005;58:216.
7. Wiesberg TT, Differding JA, Enomoto TM, Sawai RS, Muller PJ, **Schreiber MA**. Resuscitation with Lactated Ringer's (LR) vs. Normal Saline (NS) Leads to a Hypercoagulable State in an Uncontrolled Hemorrhagic Shock Swine Model. *Journal of Trauma*. 2006;59:1538.
8. Englehart M, Tieu B, Kiraly L, Sawai R, **Schreiber MA**. The Influence of Anesthesia on a Swine Model of Hemorrhagic Shock. *Shock*. 2006;25:58.

9. Tieu B, Enomoto TM, Phillips C, Hagg D, Wiesberg T, Muller P, **Schreiber MA**. Resuscitation with Normal Saline (NS) Increased Volume Requirement, Increased Cardiac Output and Decreased Systemic Vascular Resistance compared to Lactated Ringers (LR) in an Uncontrolled Hemorrhagic Shock Model in Swine. *Journal of the American College of Surgeons*. 2006;32:S32.
10. Kiraly LN, Sawai RS, Watters JM, Differding JA, Wiesberg TT, Muller PJ, **Schreiber MA**. 7.5% Saline with Dextran Resuscitation Causes Dysfunctional Inflammation in Uncontrolled Hemorrhagic Shock. *Journal of Trauma*. 2006;61:1559.
11. Englehart MS, Kiraly LN, Tieu BH, Sawai RS, Underwood SA, Muller PJ, **Schreiber MA**. Ketamine-Based Total Intravenous Anesthesia (TIVA) is Superior to Isoflurane (ISO) in a Swine Model of Hemorrhagic Shock. *Journal of Trauma*. 2006;61:1559.