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Effect of Fluid Resuscitation Strategy during En Route Care on Acute Lung Injury after Hemorrhage and Burn Injury

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14. ABSTRACT

INTRODUCTION: The epidemiology of casualties requiring Critical Care Air Transport Team (CCATT) evacuation has shifted to most of the patients suffering from multiple injuries. These injuries consist of injury of blast leading to critically ill patients suffering from total body surface area burns and high injury severity scores.
This combination of injury patterns leads to unanswered questions and contradictions regarding resuscitation strategies for patients suffering hemorrhage and burn injury. As an example, the current clinical practice guideline (CPG) for resuscitation of burn injury recommends large volumes of crystalloid resuscitation while the CPG for damage control resuscitation recommends the aggressive use of blood products and fresh frozen plasma (FFP). To further complicate resuscitation strategies in the hospital setting the predominating resuscitation strategy is the use of normal saline. These differences in CPGs and practice patterns are important to patient care, as crystalloid use is associated with worsened lung injury after trauma. Increased FFP use has been shown to be an independent risk factor in the development of lung injury in burn patients, but inadequate correction of coagulopathy with FFP in hemorrhage is associated with worsened mortality in CCATT patients. Our study focused on these inconsistencies with the intent to generate data to inform and guide decision making. **RESULTS:** Our study pursued three aims **1) Determine the optimal fluid resuscitation strategy for initial care after hemorrhage and burn injury**. Based on our data, we conclude that a combined resuscitation approach of direct peritoneal resuscitation (DPR) with normal saline (NS) followed by intravascular blood products is superior in the setting of concomitant burn injury and hemorrhagic shock. Although survival is similar between intraperitoneal strategies, the use of NS resulted in decreased systemic inflammation and blunted the onset of acute lung injury. **2) Determine the optimal fluid resuscitation strategy for hemorrhage and burn injury during simulated tactical critical care evacuation team (TCCET) movement.** Based on our data resuscitation with lactated ringers (LR) results in increased lung leukocyte infiltrate and levels of keratinocyte chemoattractant (KC) in mice who underwent a burn/hemorrhage injury plus hypoxia. The addition of hypoxia results in increased leukocyte infiltrate and KC levels when compared to normoxia regardless of resuscitation strategy. These findings suggest that resuscitation using fresh blood in 1:1 ratio potentially mitigates lung inflammation after combined burn/hemorrhage injury followed by hypoxia. **3) Determine the optimal fluid resuscitation strategy for hemorrhage and burn injury during simulated CCATT movement.** We found that, after combined burn and hemorrhage injury followed by simulated altitude 2 hours post-injury, no single resuscitation strategy demonstrates superiority when compared to baseline counterparts. Delaying simulated aeromedical evacuation to 24 hours post-injury allowed for recovery from the acute injury and demonstrated decreased acute lung injury among all resuscitation strategies. These findings suggest that a specific resuscitation strategy does not mitigate lung inflammation after combined burn and hemorrhage injury followed by simulated altitude.
CONCLUSION: Based on our data, we conclude the following: **1)** Following combined burn and hemorrhagic shock, direct peritoneal resuscitation with either NS or a peritoneal dialysis solution resulted in improved survival compared to mice receiving only crystalloid or a one-to-one transfusion of packed red blood cells (pRBCs) and FFP alone. **2)** While DPR with Delflex® 2.5 (2.5% dextrose peritoneal dialysis) reduced intestinal ischemia, intraperitoneal NS was associated with a reduced inflammatory response and delayed progression of acute lung injury. **3)** Resuscitation with LR results in increased lung leukocyte infiltrate and levels of KC in mice who underwent a burn/hemorrhage injury plus hypoxia**. 4)** The addition of hypoxia results in increased leukocyte infiltrate and KC levels when compared to normoxia regardless of resuscitation strategy. **5)** Resuscitation using fresh blood in 1:1 ratio potentially mitigates lung inflammation after combined burn/hemorrhage injury followed by hypoxia. **6)** After combined burn and hemorrhage injury followed by simulated altitude 2 hours post-injury no single resuscitation strategy demonstrates superiority when compared to baseline counterparts. **7)** Delaying simulated aeromedical evacuation to 24 hours post-injury allows for recovery and shows decreased acute lung injury among all resuscitation strategies. **8)** These findings suggest that a specific resuscitation strategy does not mitigate lung inflammation after combined burn and hemorrhage injury followed by simulated altitude. **9)** Further studies are needed to identify differences in late outcomes based on the intraperitoneal fluid used and refine the overall resuscitation strategy. Other future studies should focus on altering signaling pathways to attenuate lung injury. Additional studies should focus on the unique inflammatory response pattern that occurs in combined burn/hemorrhage injury followed by hypobaric hypoxia.

15. SUBJECT TERMS
Hemorrhage, resuscitation, CCATT, Burn, lung injury, shock

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1.0 BACKGROUND

Enemy use of improvised explosive devices as well as advanced initial care and survival of combat casualties has led to a shift in the epidemiology of casualties requiring Critical Care Air Transport Team (CCATT) evacuation, with the majority of patients suffering from multiple injuries [1]. In these patients, the most common mechanism of injury is blast [1], frequently leading to critically ill patients suffering from both significant total body surface area (TBSA) burns and high injury severity scores[2].

Lung injury, clinically defined as acute respiratory distress syndrome (ARDS) and defined in the pre-clinical setting as acute lung injury (ALI) is common in patients who suffer from burn injury[3] or need initial transfusion of more than 5 units of packed red blood cells (pRBCs) for hemorrhage [4] and is associated with a 33% mortality [3, 5, 6]. The prevention of ALI remains an opportunity for improvement during initial combat casualty and subsequent tactical and strategic evacuation.

Although combat casualty care experience and research has led to many recent advances, unanswered questions and contradictions in resuscitation strategies remain for patients suffering from hemorrhage and burn injury. For instance, the current clinical practice guidelines (CPG) for resuscitation of burn injury continues to recommend large volumes of crystalloid resuscitation [7] while the CPG for damage control resuscitation recommends the aggressive use of blood products and fresh frozen plasma (FFP) [8]. In practice, it appears that resuscitation of casualties is inconsistent, with the use of normal saline predominating in the hospital setting [9]. These differences in CPGs and practice patterns are important to patient care, as crystalloid use is associated with worsened lung injury after trauma [10-12]. Increased FFP use has been shown to be an independent risk factor in the development of lung injury in burn patients [3], but inadequate correction of coagulopathy with FFP in hemorrhage is associated with worsened mortality in CCATT patients [13].

In order to generate data to inform these issues and guide decision making, we pursued experiments based on three specific aims:

Specific aim 1: Determine the optimal fluid resuscitation strategy for initial care after hemorrhage and burn injury.

Specific aim 2: Determine the optimal fluid resuscitation strategy for hemorrhage and burn injury during simulated TCCET movement.

Specific aim 3: Determine the optimal fluid resuscitation strategy for hemorrhage and burn injury during simulated CCATT movement.

The data from these experiments has increased our understanding of the optimal resuscitation strategy after combined burn injury and hemorrhagic shock.

2.0 METHODS

We have described our methods for these experiments in a recent publication in *Military Medicine*. The following is reproduced and adapted from that manuscript [14].

Animal Model

Mice were purchased from Jackson Laboratories (Bar Harbor, ME). All experiments utilized 8 to 10-week-old male C57BL/6 mice. Housing was provided by the Laboratory Animal Medical Services facility, which consisted of a climate controlled environment with daily light-dark cycles. Mice were fed a diet of standard chow and water ad libitum and were allowed to acclimate for 1 week prior to experimentation. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. The study protocol was reviewed and approved by the University of Cincinnati Institutional Animal Care and Use Committee and the Air Force Medical Support Agency Office of Research Oversight and Compliance. Animals were handled and studies were conducted under a program of animal care accredited by AAALAC International and in accordance with the National Research Council's 2011 Guide for the Care and Use of Laboratory Animals (in compliance with Department of Defense Instruction 3216.1).

Murine Model for Burn Injury

Mice were subjected to a full-thickness scald injury adapted from models previously described [15]. Mice were anesthetized with 0.1 mg/kg pentobarbital via intraperitoneal injection. A hair clipper was used to remove hair from their dorsal surface and mice were then placed to expose 28% total body surface area. While in the template, the mice were submerged in 90.0°C water for 7 seconds to produce a full-thickness scald injury to the exposed dorsal skin. DPR was then given with 0.056 mL/g sterile normal saline or 2.5% dextrose peritoneal dialysis (Delflex) solution (Fresenius Medical Care, Waltham, MA) administered via intraperitoneal injection. Sham burn mice received the same treatment except they were immersed in room temperature water.

Murine Model for Hemorrhagic Injury

Following burn injury, mice underwent hemorrhagic shock as previously described [16]. Immediately following burn injury, the left femoral vessels were exposed and a catheter was introduced into the femoral artery. Mice were placed on a circulating water heating blanket and connected to continuous hemodynamic monitoring (Harvard Apparatus, Holliston, MA). Following cannulation, systolic blood pressure was allowed to equilibrate for 10 minutes, then blood was withdrawn from the catheter until a mean arterial blood pressure of 25 mmHg was achieved and maintained for 30 minutes. Mice were then resuscitated to a targeted systolic blood pressures of 80 mmHg over 15 minutes with either sterile lactated Ringer's (LR) solution or a balanced ratio of fresh pRBCs and FFP. Mice were allowed to recover under a heat lamp and survival was followed for 24 hours post-resuscitation. Sham-hemorrhage animals underwent femoral artery cannulation, but no blood was withdrawn and no resuscitation given. We tested the following resuscitation strategies: LR solution, balanced pRBCs and FFP (1:1), 1.5 mL intraperitoneal normal saline (NS) followed by intravascular LR solution (NS +LR), 1.5 mL intraperitoneal NS followed by balanced pRBCs and FFP (NS + 1:1), 1.5

mL intraperitoneal Delflex followed by intravascular LR (D + LR), and 1.5 mL intraperitoneal Delflex followed by balanced pRBCs and FFP (D + 1:1).

Blood Banking
Mice were anesthetized with 0.1 mg/kg pentobarbital, whole blood was collected, and pRBCs and FFP were prepared as we have previously described [17].

Electrolyte and Serum Evaluation
At 1 and 4 hours post-resuscitation, blood samples were obtained via cardiac puncture after intraperitoneal pentobarbital anesthesia. Whole blood samples were analyzed with an iSTAT (Abaxis, Union City, CA) to determine hemoglobin, hematocrit, blood urea nitrogen, glucose, chloride, sodium, potassium, pH, partial pressure of carbon dioxide, bicarbonate, anion gap, and base excess. Serum was collected after centrifugation (8000 rpm for 10 minutes) of whole blood, then analyzed by a cytometric bead array (BDBiosciences, San Jose, CA) for quantification of interleukin 6 (IL-6), IL-10, tumor necrosis factor alpha (TNFα), and macrophage inflammatory protein 1 alpha (MIP-1α). Intestinal injury was determined using intestinal fatty acid binding protein (IFABP) enzyme-linked immunosorbent assay (MyBioSource, San Diego, CA).

Histology
Lung tissue was harvested at 1 hour and 4 hours post-resuscitation. The trachea was flushed immediately with neutral buffered formalin then fixed in paraffin. Slices of lung parenchyma were mounted on slides and stained with hematoxylin and eosin. Injury was quantified using the Lung Injury Scale as has been described previously [18, 19]. Five random areas of each lung sample were analyzed under 20x magnification and scored based on the five following categories: septal thickness, alveolar edema, hyaline membranes, inflammatory cell infiltrate, and small airway epithelial injury. Each category was scored based on severity from 0 to 5 and then averaged to calculate a total score.

Hypoxia and hypobaric models
To simulate brief hypoxia in a similar fashion to that as occurs during TCCET movement, following 30 minutes of recovery, mice were placed in a hypoxia chamber with 15.4% FiO2 for 30 minutes to simulate tactical aeromedical evacuation. Mice were euthanized after 1 hour for tissue histology and cytokine analysis. Lung histology was evaluated using Ly6G staining for leukocyte infiltration.

To examine the effect of hypobaric hypoxia, mice underwent a combined burn and hemorrhage with resuscitation, then were allowed to recover for either 2 hours or 24 hours to simulate immediate and delayed evacuation. In order to simulate CCATT movement, mice were then exposed to simulated altitude at 8800 ft above sea level (via altitude chamber) for 2 hours. Mice were euthanized immediately upon removal from the chamber for tissue histology and cytokine analysis.

Statistical Analysis
Quantitative results are reported as mean ± standard error of the mean. Two-tailed Student's t-test was used to analyze continuous variables. Survival was compared using the Mantel-Cox log-rank test. A p-value of less than 0.05 was considered statistically significant.

3.0 RESULTS

Specific aim 1: Determine the optimal fluid resuscitation strategy for initial care after hemorrhage and burn injury.

Data generated under this aim is presented in a recent publication in *Military Medicine*. The following is reproduced and adapted from that manuscript [14].

Survival

Survival data are displayed in **Figure 1.**

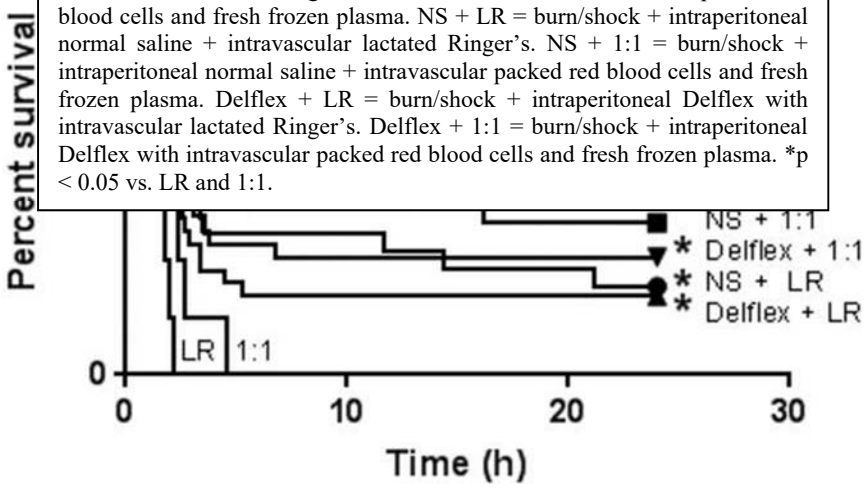

FIGURE 1. Kaplan-Meier survival curve. Mice underwent sham injury or injury and survival was determined over 24 hours. LR = burn/shock + intravascular lactated Ringer's. 1:1 = burn/shock + intravascular packed red blood cells and fresh frozen plasma. NS + LR = burn/shock + intraperitoneal normal saline + intravascular lactated Ringer's. NS + 1:1 = burn/shock + intraperitoneal normal saline + intravascular packed red blood cells and fresh frozen plasma. Delflex + LR = burn/shock + intraperitoneal Delflex with intravascular lactated Ringer's. Delflex + 1:1 = burn/shock + intraperitoneal Delflex with intravascular packed red blood cells and fresh frozen plasma. *p < 0.05 vs. LR and 1:1.

One hundred percent mortality was demonstrated in mice subjected to combined burn and hemorrhagic shock that were resuscitated with either LR or 1:1 pRBCs to FFP alone, with median survival times of 1.47 (interquartile range (IQR) 1.37 – 1.89) hours and 2.08 (IQR 1.63 – 2.51) hours, respectively (p = not significant). The addition of direct intraperitoneal resuscitation to standard resuscitation with LR or blood products was associated with a significant improvement in survival after injury (24-hour survival of 21.7% [median survival 2.93 hours; IQR 2.41 – 17.78 hours] for NS + LR, $p < 0.01$ vs. LR; 37.5% survival [median survival 5.54 hours; IQR 2.89 – 24.00 hours] for NS + 1:1, $p < 0.01$ vs. 1:1). No significant difference in survival was seen between NS + LR and NS + 1:1 groups at 24 hour. Direct intraperitoneal resuscitation with Delflex solution also resulted in a significant mortality improvement as compared to mice receiving no intraperitoneal resuscitation (24-hour survival of 19.36% [median survival 2.45 hours; IQR 1.68 – 3.9 hours] for D + LR, $p < 0.01$ vs. LR; 29.03% survival [median survival 2.40 hours; IQR 1.63 – 24 hours] for D + 1:1, $p < 0.01$ vs. 1:1). No significant differences in survival were seen after DPR with normal saline or Delflex solution.

Hemodynamics during Hemorrhagic Shock

Mean blood volume withdrawn to achieve hemorrhagic shock and mean volume of intravascular fluid required to achieve goal blood pressure during resuscitation were determined. This data is presented in the Supplemental Data (**Figure 2**).

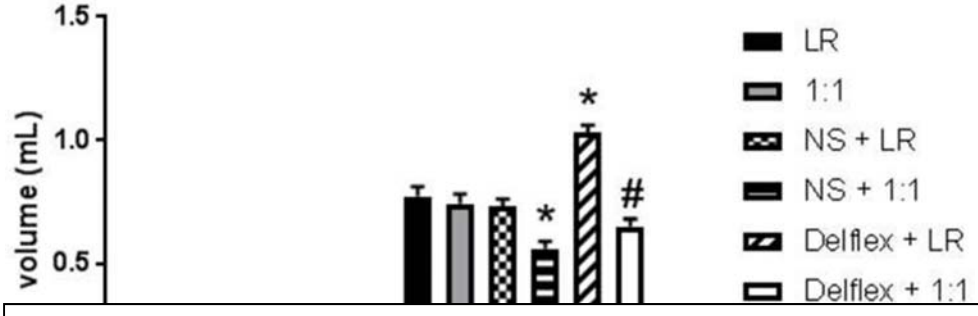

FIGURE 2. Volume withdrawn and needed for resuscitation. Mice were subjected to burn injury followed by hemorrhagic shock, then resuscitated. Left: There were no significant differences in volume of blood removed to achieve target blood pressure between all groups. Right: Intravascular volume required to achieve target systolic blood pressure; *p < 0.05 vs. all groups, #p < 0.05 vs. all groups except LR.

There was no difference in the amount of blood removed for each group. Mice resuscitated with intraperitoneal NS plus intravascular 1:1 required the least amount of intravascular volume to achieve target blood pressure (0.56 ± 0.03 mL, $p < 0.05$ vs. all groups). Mice that received intraperitoneal Delflex plus LR required the most intravascular volume to achieve target blood pressures compared to all other groups (1.03 ±0.03 mL, $p < 0.01$ vs. all groups). Delflex + 1:1 had variable effects on the total intravascular volume required. It reduced the total intravascular resuscitation volume compared to Delflex + LR (0.65 ± 0.03 mL, $p < 0.01$), required greater volume compared to NS + 1:1 ($p = 0.03$), and was similar to NS + LR, LR alone, and 1:1 alone (0.72 ± 0.03 mL, $p = 0.07$; 0.77 ± 0.04 mL, $p = 0.05$; 0.74 ± 0.04 mL, $p = 0.12$, respectively).

Electrolyte Analysis

We analyzed whole blood in mice euthanized 1h post-resuscitation. Only hct and hgb significantly differed between the NS + LR and the NS + 1:1 groups ($p = 0.01$). Similarly, hct and hgb were the only significant differences between Delflex + LR and Delflex + 1:1 ($p < 0.05$). Resuscitation with Delflex + 1:1 resulted in worse metabolic acidosis when compared to NS + 1:1 ($p < 0.05$), but this difference was not seen in comparison to the other groups (**Table 1).**

Serum Analysis

Serum collected from mice was analyzed for cytokine concentrations, and the results for selected cytokines are shown in **Figure 3**.

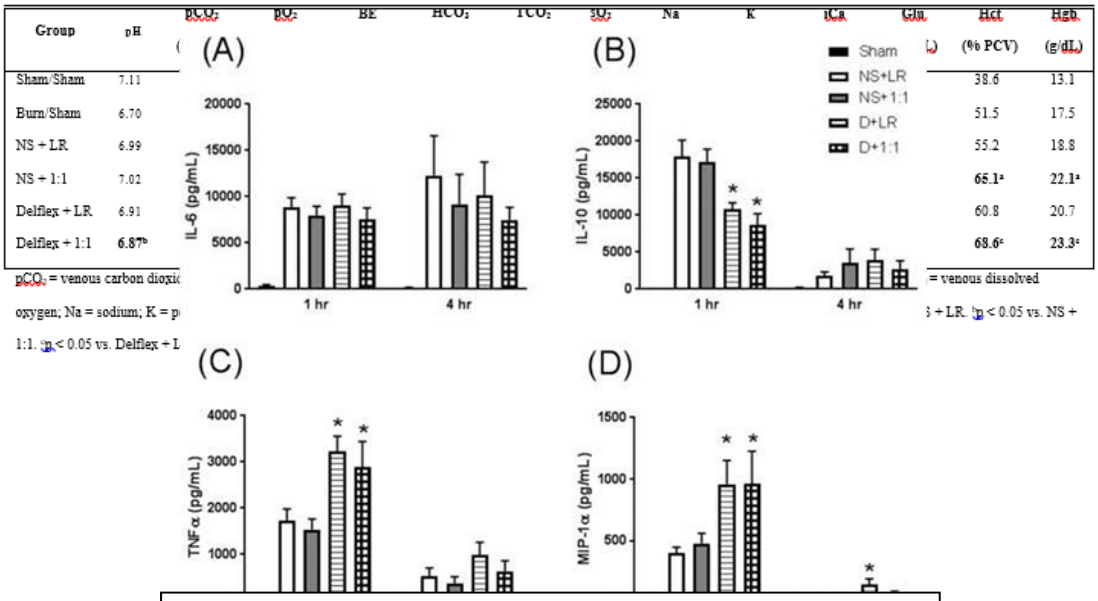

FIGURE 3. Serum cytokine concentrations. Mice were subjected to sham injury or burn/shock and resuscitated with either NS + LR, NS + 1:1, Delflex + LR, or Delflex + 1:1. (A) Serum IL-6 concentrations at 1 and 4 hours post-resuscitation. (B) Serum IL-10 concentrations at 1 and 4 hours post-resuscitation. *$p < 0.05$ vs sham, NS + LR, and NS + 1:1. (C) Serum TNFα at 1 and 4 hours post-resuscitation. *$p < 0.05$ compared to sham, NS + LR, and NS + 1:1. (D) MIP-1α at 1 and 4 hours post-resuscitation. *$p<0.05$ vs sham, NS + LR, and NS + 1:1 at 1 hour and *$p<0.05$ vs. all 4 hour groups.

There were no significant differences in cytokine levels between mice resuscitated with NS + LR and NS + 1:1 at either 1 or 4 hours after resuscitation. Mice resuscitated with intraperitoneal Delflex had significantly lower levels of IL-10 ($p < 0.05$) and increased levels of both TNFα and MIP-1α ($p < 0.05$). There was no significant difference between Delflex + LR and Delflex + 1:1. Serum MIP-1a remained elevated in the Delflex + 1:1 mice at 4 hours (Figure 3D).

Intestinal fatty acid binding protein (IFABP) levels are shown in **Figure 4**.

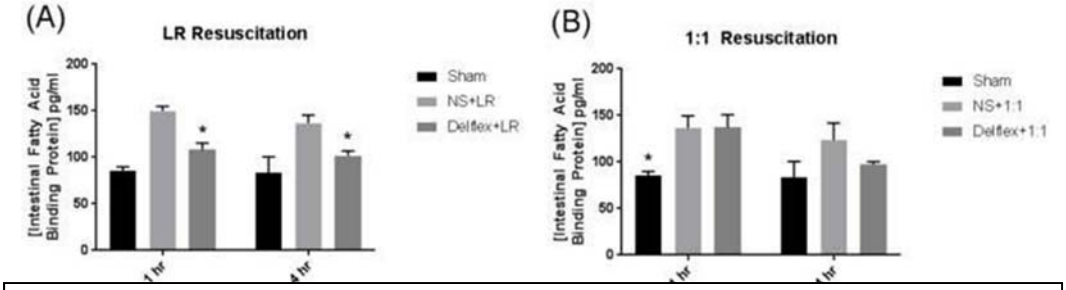

FIGURE 4 . IFABP serum concentrations 1 and 4 hours post-resuscitation. Mice were subjected to sham injury or burn/shock and resuscitated with either NS + LR, NS + 1:1, Delflex + LR, or Delflex + 1:1. *p < 0.05 vs. all other groups.

Mice resuscitated with Delflex + LR demonstrated significantly lower levels of serum IFABP when compared to mice resuscitated with NS + LR at both 1 hour and 4 hours following resuscitation ($p < 0.05$, Figure 3). This difference was not seen in mice resuscitated with fresh pRBCs and FFP (Figure 4B).

Histology

Representative images of lung histology are displayed in **Figure 5**. Mice resuscitated with direct intraperitoneal NS had lower lung injury severity scores compared to those that received intraperitoneal Delflex at 1 hour (NS + LR: 2.63 ± 1.54 points; NS + 1:1: 1.86 ± 0.29 points; Delflex + LR: 11.73 ± 0.98 points; Delflex + 1:1: 11.33 ± 0.34 points; $p < 0.01$). This difference no longer existed at 4 hours. There was no significant difference between intravascular LR and 1:1 within each direct intraperitoneal resuscitation group.

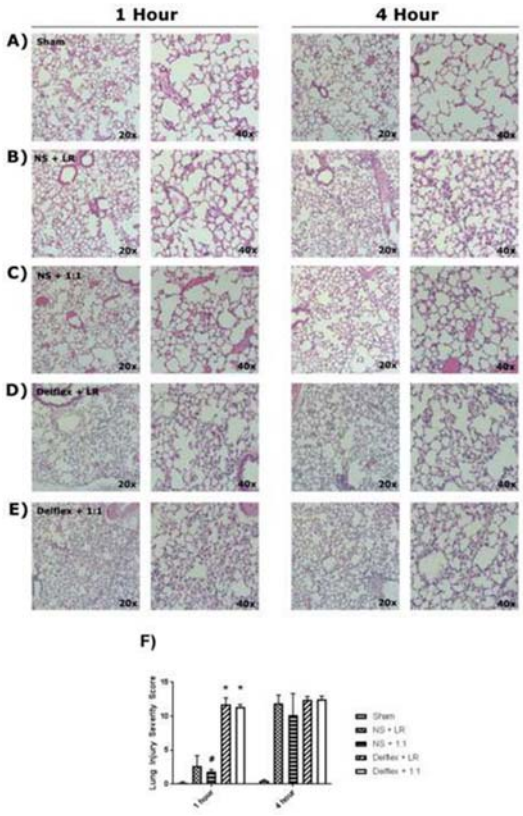

FIGURE 5. Lung histology. Mice were subjected to burn/shock and resuscitated with (A) sham, (B) NS + LR, (C) NS + 1:1, (D) Delflex + LR, or (E) Delflex + 1:1. Whole lung tissue was harvested 1 and 4 hours post-resuscitation and fixed in neutral buffered formalin. Tissue was mounted in paraffin and stained with hematoxylin and eosin. Under 20x magnification, lung injury was assessed using the Lung Injury Score and the mean score was determined for each sample. (F) Graphic representation of the Lung Injury Score. *p < 0.05 vs. vs sham, NS + LR, and NS + 1:1. #p < 0.05 vs. sham.

Specific aim 2:

Determine the optimal fluid resuscitation strategy for hemorrhage and burn injury during simulated TCCET movement.

Data generated from these experiments has been submitted for consideration for presentation at upcoming national meetings. We anticipate that this work will result in a manuscript.

We sought to determine the optimal resuscitation strategy after combined burn and hemorrhage injury followed by hypoxia. Male mice underwent a 15% TBSA burn (all mice received 1.5 mL intraperitoneal saline post burn) followed by femoral artery cannulation and hemorrhage to systolic blood pressure of 25 mmHg +/- 5 mmHg. After 30 minutes of hemorrhage, mice were either resuscitated with lactated Ringer's solution (LR), fresh pRBCs and plasma in a 1:1 ratio (1:1), or 14 day old aged pRBCs with plasma in a 1:1 ratio (14 day). Following 30 minutes of recovery, mice were placed in a hypoxia chamber with 15.4% FiO2 for 30 minutes to simulate tactical aeromedical evacuation. Mice were euthanized after 1 hour for tissue histology and cytokine analysis. Lung histology was evaluated using Ly6G staining for leukocyte infiltration.

When compared with mice receiving either fresh or 14 day 1:1, mice receiving LR resuscitation demonstrated increased leukocyte infiltrate on histologic evaluation ($p<0.05$; **Figure 6**).

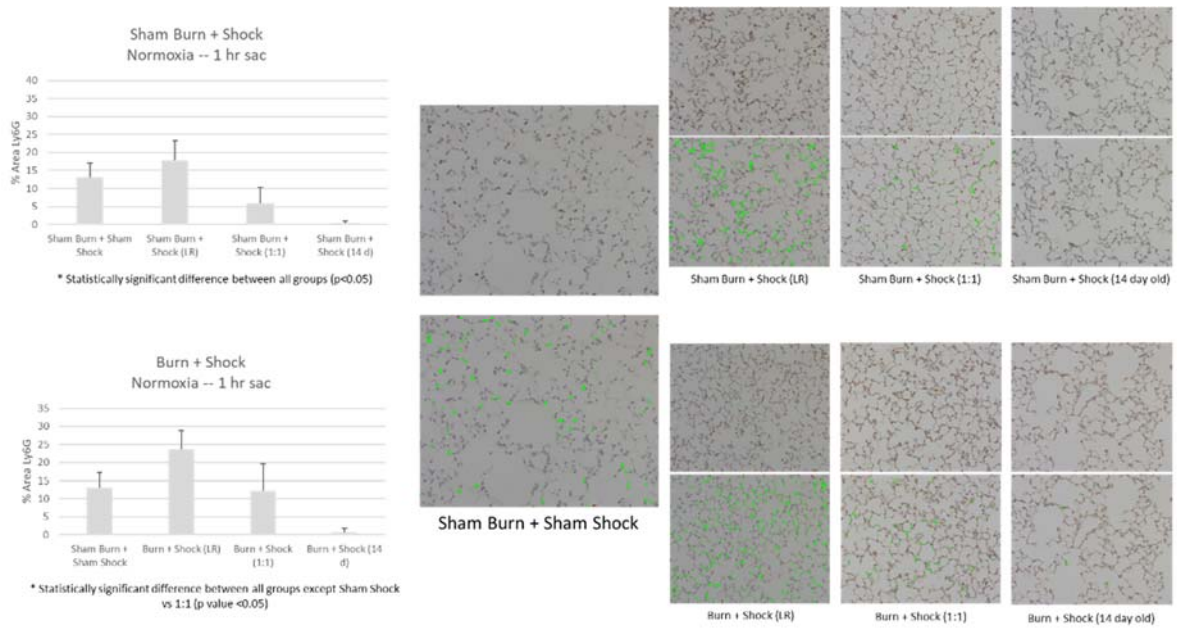

FIGURE 6. Lung histology as well as staining for leukocytes (green) in mice treated with sham burn, burn, sham shock, and shock, then normoxia. LR resuscitation resulted in the highest degree of leukocyte infiltration into the lung.

When these experiments were repeated and mice were subjected to 30 minutes of hypoxia following resuscitation, we found that LR again resulted in the highest degree of pulmonary leukocyte infiltration. This data is presented in **Figure 7.**

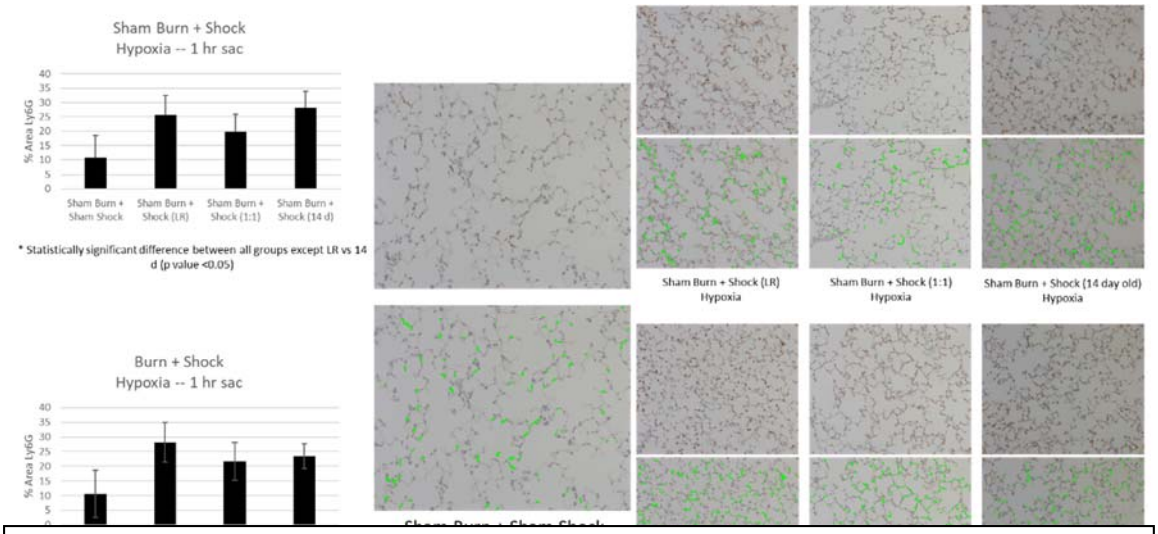

FIGURE 7. Lung histology as well as staining for leukocytes (green) in mice treated with sham burn, burn, sham shock, and shock, then brief hypoxia. LR resuscitation resulted in the highest degree of leukocyte infiltration into the lung.

The hypoxia groups demonstrated increased leukocyte infiltrate among all resuscitation strategies when compared to their normoxia counterparts ($p<0.05$).

When we compared the data from mice subjected to sham burn, burn, sham shock, and shock with either normoxia or hypoxia, we found that hypoxia after injury resulted in increased pulmonary leukocyte infiltration in all resuscitation strategies (**Figure 8**).

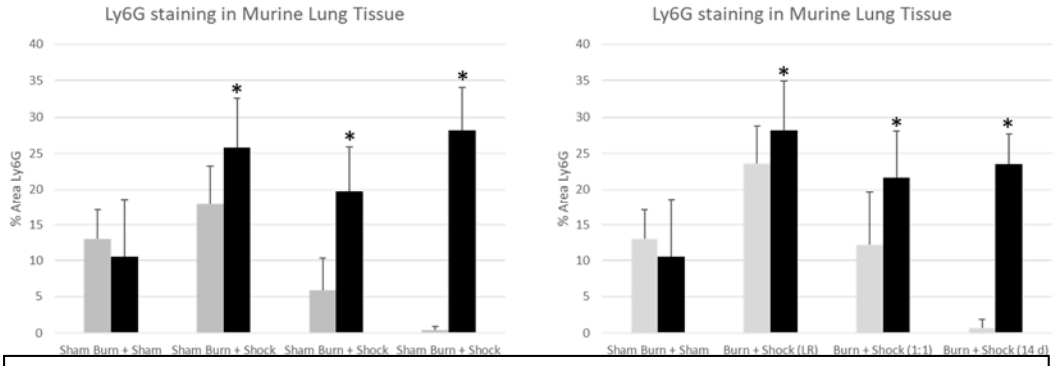

FIGURE 8. Lung histology analysis for mice treated with sham burn, burn, sham shock, and shock followed by either normoxia (gray bars) or hypoxia (black bars). Hypoxia resulted in increased lung inflammation in all resuscitation groups (*$p<0.05$ vs corresponding normoxia group).

When we analyzed serum cytokine and chemokine levels from these mice, we found that the serum KC (the mouse homologue of IL-8) was elevated after resuscitation with aged pRBCs. This was exacerbated in mice that were then treated with hypoxia (data not shown). Serum levels of the chemokine MIP-1 alpha were also elevated after hypoxia in sham treated mice and mice subjected to hemorrhage alone. There were generally no significant differences in between normoxia and hypoxia after combined injury, but there were increased serum MIP-1 alpha levels after resuscitation with aged blood (data not shown). We also found elevated serum levels of the chemokine MDC after hemorrhage and resuscitation with aged blood followed by hypoxia. This difference did not persist when burn injury was added (data not shown). In contrast, we found a several-fold increase in serum MIP-2 (a murine homologue of IL-8) when animal subjected to hemorrhage and resuscitation with either LR or fresh 1:1 were treated with hypoxia (data not shown).

When we analyzed lung tissue for cytokines and chemokines from mice that were treated with hemorrhage with and without burn injury, then either normoxia or hypoxia, we found that lung KC levels were significantly increased in mice treated with burn, hemorrhage, and hypoxia and resuscitated with LR, 1:1, or aged pRBCs (**Figure 9**).

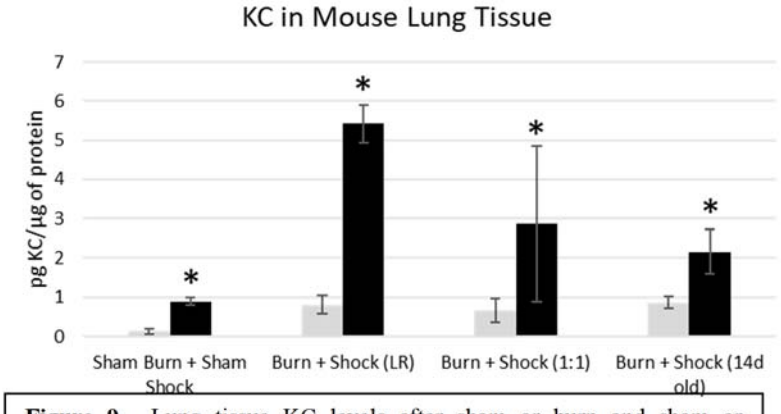

Figure 9. Lung tissue KC levels after sham or burn and sham or hemorrhage followed by hypoxia. * $p<0.05$ vs normoxia groups.

These differences were less pronounced with pulmonary MIP-1 alpha, MDC, and IL-6 (data not shown). This pattern was also seen, but to a lesser extent, with pulmonary MIP-2 (data not shown). Attempts to mitigate lung injury by utilizing a blocking antibody against KC were not successful.

Specific aim 3: Determine the optimal fluid resuscitation strategy for hemorrhage and burn injury during simulated CCATT movement.

Data generated from these experiments has been submitted for consideration for presentation at upcoming national meetings. We anticipate that this work will result in a manuscript.

Our goal with this series of experiments was to determine the optimal resuscitation strategy in a model of combined burn/hemorrhage injury followed by exposure to hypobaric hypoxia. Male mice underwent a combined burn and hemorrhage with 15% TBSA burn injury (all mice received 1.5 mL intraperitoneal normal saline post burn) followed by femoral artery cannulation and hemorrhage to systolic blood pressure of 25 mmHg +/- 5 mmHg. After 30 minutes of hemorrhage, mice were either resuscitated with lactated Ringer's solution (LR), fresh pRBCs and plasma in a 1:1 ratio (Fresh 1:1), or 14 day old aged pRBCs and plasma in a 1:1 ratio (14 day 1:1). Mice were decannulated and allowed to recover for either 2 hours or 24 hours to simulate immediate and delayed evacuation. In order to simulate CCATT mission, mice were then exposed to simulated altitude at 8800 ft above sea level (via altitude chamber) for 2 hours. Mice were euthanized immediately upon removal from the chamber for tissue histology and cytokine analysis. Lung histology was evaluated using Ly6G staining for leukocyte infiltration and quantified.

When compared with mice receiving either LR or fresh 1:1, mice receiving 14 day 1:1 resuscitation after combined injury and simulated aeromedical evacuation demonstrated increased pulmonary inflammatory cell infiltrate on histologic evaluation (p-value <0.05). When compared to baseline controls no resuscitation strategy demonstrated superiority when evaluating pulmonary leukocyte infiltration (**Figure 10**).

Evaluating simulated evacuation 2 hours post-injury versus 24 hours post-injury reveal that there was consistently decreased leukocyte infiltration among all groups **(Figure 11).**

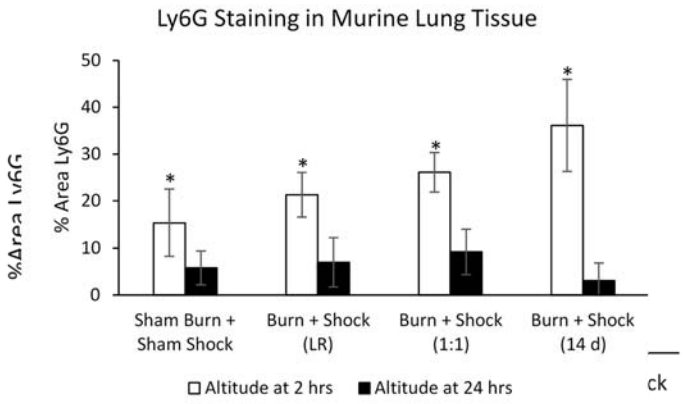

Figure 11: Leukocyte infiltration by (%) area of Ly6G staining after combined injury followed by simulated aeromedical evacuation at 2 or 24 hours after injury. * p<0.05 vs no altitude groups.

after combined injury followed by simulated aeromedical evacuation, * p<0.05 vs no altitude groups.

Tissue cytokine analysis demonstrated no statistical differences between resuscitation groups that underwent simulated evacuation at 2 hours post-injury in terms of IL-10, IL-1 beta, IL-6, MCP-1, MDC, MIP-1 alpha, MIP-2, KC, IFN gamma, or RANTES.

4.0 DISCUSSION

In the present series of studies, we investigated resuscitation strategies following combined burn injury and hemorrhage. The different resuscitation strategies examined included LR, 1:1 FFP to pRBCs with freshly donated pRBCs, and 1:1 FFP to pRBCs with 14 day old pRBCs. In addition, we initially carried out a series of experiments that examined the need for direct peritoneal resuscitation in addition to the above outlined resuscitation studies.

In our first series of experiments, we found that mice resuscitated with either LR or equal volume of pRBCs and FFP demonstrated a median survival time of 1.47 hours and 2.08 hours, respectively, following combined burn and hemorrhagic injury. The use of intravascular crystalloid as compared to blood products did not provide a survival benefit.

In contrast, a significant survival advantage was seen with the addition of direct peritoneal resuscitation (DPR) with either NS or a peritoneal dialysis solution to either crystalloid or blood product resuscitation. Intraperitoneal NS or Delflex did not provide a significant survival advantage over each other, but the use of NS resulted in decreased aspects of the systemic inflammatory response and decreased early lung

inflammation. Interestingly, the use of intraperitoneal Delflex resulted in a decreased concentration of serum intestinal fatty acid binding protein, suggesting decreased intestinal damage following burn and hemorrhage. As expected, mice that received blood products had improvements in hematocrit and hemoglobin at 1 hour. Mice that received intraperitoneal Delflex demonstrated a more severe metabolic acidosis at 1 hour, especially in conjunction with 1:1 ratios of pRBCs to FFP.

In the setting of a combined burn injury and hemorrhagic shock, our data supports a blended resuscitation, with direct peritoneal resuscitation combined with intravascular resuscitation. In the first series of experiments, mice resuscitated with blood products required less volume than those that only received crystalloid. The effect was most dramatic in mice receiving intraperitoneal NS followed by intravascular pRBCs and FFP. This strategy also delayed the onset of acute lung injury when compared to the intraperitoneal Delflex groups. The benefit of avoiding fluid overload is consistent with other literature studying both murine and human models. Thus, based on our data, we conclude that a combined resuscitation approach of DPR with NS followed by intravascular blood products is superior in the setting of concomitant burn injury and hemorrhagic shock. Although survival is similar between intraperitoneal strategies, the use of NS resulted in decreased systemic inflammation and blunted the onset of acute lung injury.

Our second series of studies examined the outcomes of different resuscitation strategies in the combined model followed by brief hypoxia. We found that resuscitation with LR results in increased lung leukocyte infiltrate and levels of KC in mice who underwent a burn/hemorrhage injury plus hypoxia. The addition of hypoxia results in increased leukocyte infiltrate and KC levels when compared to normoxia regardless of resuscitation strategy. These findings suggest that resuscitation using fresh blood in 1:1 ratio potentially mitigates lung inflammation after combined burn/hemorrhage injury followed by hypoxia.

Our third series of experiments investigated the outcomes of different resuscitation strategies in the combined model followed by hypobaric hypoxia. We found that, after combined burn and hemorrhage injury followed by simulated altitude 2 hours post-injury, no single resuscitation strategy demonstrates superiority when compared to baseline counterparts. Delaying simulated aeromedical evacuation to 24 hours post-injury allowed for recovery from the acute injury and demonstrated decreased acute lung injury among all resuscitation strategies. These findings suggest that a specific resuscitation strategy does not mitigate lung inflammation after combined burn and hemorrhage injury followed by simulated altitude.

When evaluating our data, several limitations must be considered. Due to logistic and technical limitation, our model of injury is sequential, rather than simultaneous. All efforts are made to minimize the time between burn and hemorrhage, but 10 minutes of time is needed for femoral artery cannulation. This delay could affect aspects of the results. This study also lacks data on long-term outcomes other than survival. Since clinically significant lung injury may not occur acutely, lack of 24-hour histology and cytokine analysis limits the strength of our conclusions. However, given low median survival times for all treatment groups, investigating cytokine concentrations and histology at 24 hours would introduce a profound survivor bias. In addition, these experiments took place in mice. While aspects of the model recapitulate the human condition, our findings may not apply across species. Lastly, we transfused blood in components, but recent data are suggesting that fresh whole blood may be the superior resuscitation fluid in the setting of hemorrhagic shock.

5.0 CONCLUSIONS

Based on our data, we conclude the following:

1. Following combined burn and hemorrhagic shock, direct peritoneal resuscitation with either NS or a peritoneal dialysis solution resulted in improved survival compared to mice receiving only crystalloid or a one-to-one transfusion of pRBCs and FFP alone.

2. While DPR with Delflex® reduced intestinal ischemia, intraperitoneal NS was associated with a reduced inflammatory response and delayed progression of acute lung injury.

3. Resuscitation with LR results in increased lung leukocyte infiltrate and levels of KC in mice who underwent a burn/hemorrhage injury plus hypoxia.

4. The addition of hypoxia results in increased leukocyte infiltrate and KC levels when compared to normoxia regardless of resuscitation strategy.

5. Resuscitation using fresh blood in 1:1 ratio potentially mitigates lung inflammation after combined burn/hemorrhage injury followed by hypoxia.

6. After combined burn and hemorrhage injury followed by simulated altitude 2 hours post-injury no single resuscitation strategy demonstrates superiority when compared to baseline counterparts.

7. Delaying simulated aeromedical evacuation to 24 hours post-injury allows for recovery and shows decreased acute lung injury among all resuscitation strategies.

8. These findings suggest that a specific resuscitation strategy does not mitigate lung inflammation after combined burn and hemorrhage injury followed by simulated altitude.

9. Further studies are needed to identify differences in late outcomes based on the intraperitoneal fluid used and refine the overall resuscitation strategy. Other future studies should focus on altering signaling pathways to attenuate lung injury. Additional studies should focus on the unique inflammatory response pattern that occurs in combined burn/hemorrhage injury followed by hypobaric hypoxia.

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