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Intrathoracic Pressure Regulator Performance in the Setting of Hemorrhage and Acute Lung Injury

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14. ABSTRACT

Introduction: The intrathoracic pressure regulator (ITPR) is a device designed to enhance venous return to the heart and subsequently cardiac preload by inducing negative end expiratory pressure in mechanically ventilated patients. Previous preclinical studies have shown increased mean arterial pressure (MAP) and decreased intracranial pressure (ICP) with use of the device. The aim of this study was to evaluate the hemodynamic and respiratory effects of ITPR in a porcine polytrauma model of hemorrhagic shock and acute lung injury (ALI).

Methods: Swine were anesthetized and underwent a combination of sham, hemorrhage, and/or lung injury. Our experimental groups included no injury with and without ITPR (ITPR, Sham), hemorrhage with and without ITPR (ITPR/Hem, Hem), and hemorrhage and acute lung injury with and without ITPR (ITPR/Hem/ALI, Hem/ALI). The ITPR device was started at a setting of -3 cmH_2O and incrementally decreased by 3 cmH_2O after 30 minutes on each setting with 15 minutes allowed for recovery between settings to a nadir of -12 cmH_2O. Vital signs and ventilator settings were recorded at baseline, following each injury, at the end of each respective ITPR setting, and at the end of each recovery period. Arterial blood gas measurements were obtained at baseline, following each injury, and at the end of recovery period. Of note, due to gasping (at pressures below -6 cmH_2O) with the ITPR device, all animals were chemically paralyzed.

Results: Adequate shock was induced in the hemorrhage model, with the MAP being decreased in the Hem and ITPR/Hem group compared to Sham and ITPR/Sham, respectively, at all time points (Hem 54.2 ± 6.5 mmHg vs. 88.0 ± 13.9 mmHg, $p<0.01$, -12 cmH_2O; ITPR/Hem 59.5 ± 14.4 mmHg vs. 86.7 ± 12.1 mmHg, $p<0.01$, -12 cmH_2O). In addition, the partial pressure of arterial oxygen/fraction of inspired oxygen (PaO_2/FIO_2) ratio was appropriately decreased in Hem/ALI compared to Sham and Hem groups (231.6 ± 152.5 vs. 502.0 ± 24.6 (Sham) $p<0.05$ and vs. 463.6 ± 10.2 (Hem) $p<0.01$, -12 cmH_2O). The heart rate was consistently higher in the ITPR/Hem/ALI group compared to the Hem/ALI group (255 ± 26 bpm vs. 150.6 ± 62.3 bpm, -12 cmH_2O) and higher in the ITPR/Hem group compared to Hem. The respiratory rate (adjusted to maintain pH) was also higher in the ITPR/Hem/ALI group compared to Hem/ALI at -9 and -12 cmH_2O (32.8 ± 3.0 rpm vs. 26.8 ± 3.6 rpm, -12 cmH_2O) and higher in the ITPR/Hem group compared to Hem at -6, -9, and -12 cmH_2O. The cardiac output was higher only at select settings in the ITPR/Hem and ITPR/Hem/ALI groups compared to injury controls. Lung compliance and functional residual capacity were both consistently decreased in all three ITPR groups compared to their controls. There were no consistent differences in temperature, ICP, cerebral perfusion pressure, central venous pressure, pulmonary capillary wedge pressure, tidal volumes, positive end expiratory pressure, pH, partial pressure of carbon dioxide, bicarbonate, lactic acid, sodium, potassium, calcium, hematocrit, or hemoglobin between groups.

Conclusion: In this swine polytrauma model, we demonstrate successful establishment of hemorrhage and combined hemorrhage/ALI models. While ITPR did not demonstrate a benefit for MAP or ICP, our data demonstrate that the ITPR device induced tachycardia with associated increase in cardiac output, as well as tachypnea with decreased lung compliance, functional residual capacity, and PaO_2/FIO_2 ratio. Therefore, implementation of the ITPR device in the setting of polytrauma may compromise pulmonary function without significant hemodynamic improvement.

15. SUBJECT TERMS

Intrathoracic Pressure Regulator, Polytrauma, Porcine

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INTRODUCTION

Hemorrhage is one of the leading causes of preventable death following traumatic injury in both civilian and military populations. [1-3] The majority of nonsurvivable casualties on the battlefield die prior to ever reaching a treatment facility. [4] Hemorrhage control techniques and damage control resuscitation have improved outcomes even in these challenging prehospital environments. [5-8] The severity, diversity, and combination of injury patterns seen in recent conflicts have added to the complexity of care for these critically injured patients, leading to the use of new modalities to reduce death from hemorrhagic shock, including extremity and junctional tourniquets, injectable wound treatments, and resuscitative balloon occlusion of the aorta. [9]

Intrathoracic pressure regulation (ITPR) can be achieved utilizing a device to enhance venous return to the heart and subsequently cardiac preload by inducing NEEP in mechanically ventilated patients. The device has previously been shown to improve short-term survival in pigs that underwent an induced cardiac arrest. [10] Other preclinical trials have also shown increased mean arterial pressure (MAP) and decreased intracranial pressure (ICP) in both hypotensive pigs following hemorrhage and in normovolemic swine. [10-12] These findings have produced interest in the use of the device in patients following traumatic injury.

The ITPR device has shown improvement in MAP in preclinical hemorrhage models, but its use in polytrauma has not been investigated. ITPR might allow improved circulatory performance in the face of hypotension when methods of volume resuscitation are unavailable. In this study, we compared the use of ITPR in a swine model of isolated hemorrhage and combined hemorrhage and lung injury. Our aim was to determine whether the device could improve prehospital care provided to patients with multiple severe traumatic injuries.

METHODS

Animal Model

The Institutional Animal Care and Use Committees at the University of Cincinnati and the United States Air Force Research Oversight and Compliance Division approved all experiments. All animals were female pigs obtained from Isler Genetics (Prospect, OH). The pigs were housed in the Laboratory of Animal Medical Services facility that provides a climate-controlled environment and a 12 hour light-dark cycle. The pigs were fed standard chow and given water *ad libitum.* The pigs were acclimated to the animal husbandry environment for 2-5 days prior to the experiments.

The animals were initially sedated with an intramuscular injection of a mixture containing telazol (4-7 mg/kg), xylazine (0.1-2 mg/kg) and atropine (0.04-0.4 mg/kg). Orotracheal intubation was performed and anesthesia was initiated with inhaled isoflurane then transitioned to propofol (15-25 mg/kg/hr) following placement of all lines and monitoring devices. Peripheral intravenous catheters were placed in each ear for fluid administration. Bilateral femoral arteries were cannulated via a cut-down technique. Catheters were placed in the left femoral artery for continuous blood pressure monitoring and in the right femoral artery to allow for hemorrhage. An 8 French introducer sheath was inserted into the right internal jugular vein via a cut down technique. A 7.5 French pulmonary artery catheter (Edwards Lifesciences, Irvine, CA) was then placed to monitor mean pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output and central venous pressure.

Neurologic monitoring

Following intubation and line placement, the pigs were then placed prone on a table and the central portion of the scalp was removed to expose the cranium. A 20 mm burr hole was made in the right parietal bone, centered 16 mm anterior to the coronal suture and 12 mm lateral to the sagittal suture. Brain tissue oxygenation was monitored via a Licox probe (Integra, Saint Priest, France) placed in gray matter. In the same gyrus, a Bowman Perfusion probe (Hemedex, Cambridge, MA) was placed to monitor brain

tissue perfusion. All data was monitored and recorded by a CNS Monitor (Moberg Research INC, Ambler, PA). A 6 mm burr hole was placed in the left parietal bone, centered 16 mm anterior to the coronal suture and 10 mm lateral to the sagittal suture. The dura was opened in the 6 mm burr hole and a NEUROVENT-P intracranial pressure monitor (RAUMEDIC, Mills River, NC) was placed within the parenchyma just below the dura.

Acute Lung Injury Model

Following placement of all monitoring devices, pigs were randomized to one of six groups: Sham injury, ITPR after sham injury, Hemorrhage, ITPR after hemorrhage, ALI and hemorrhage, ITPR after ALI and hemorrhage. acute lung injury or no lung injury. With FiO2 set to 1.0, mild to moderate acute respiratory distress syndrome, defined as a PaO_2/FiO_2 ratio of < 250, was created by repeated bronchoalveolar lavage through the endotracheal tube with warm saline solution. In order to determine when the goal PaO_2/FiO_2 ratio was reached, an arterial blood gas was performed when SpO_2 was ≤ 95% after a 15-minute stabilization period. This procedure was repeated until the desired PaO_2/FiO_2 was reached

Hemorrhagic Shock Model

For animals randomized to mean arterial pressure-controlled hemorrhage, blood was withdrawn at a rate of 100 mL/min until a mean arterial pressure (MAP) of 35±5 mmHg was achieved. Hemorrhaged animals were maintained at this MAP for one hour. After the shock phase, pigs were resuscitated with intravenous crystalloid fluids until a MAP of 50±5 mmHg was achieved, to simulate prehospital resuscitation.

Intrathoracic Pressure Regulator

After induction of lung injury and shock, the VPOD intrathoracic pressure regulator (Advanced Circulatory System Inc., Roseville, MN) was attached in series between the endotracheal tube and a Carescape R860 ventilator (GE Healthcare, Chicago, IL). In addition to providing mechanical ventilation,

the R860 has the ability to measure pulmonary function and lung mechanics including end expiratory lung volume (EELV) and dead space/tidal volume ratio (V_D/V_T). EELV was determined by nitrogen washout during a step change in FiO_2. The ITPR device was started at -3 cm H_2O and decreased by an increment of -3 cm H_2O until a maximum of -12 cm H_2O was reached. Vital signs, pulmonary function measurements and neurologic monitoring parameters were recorded following 30 minutes at each device setting and following 15 minutes of recovery prior to starting the next device setting. Injury controls were established with Sham groups with and without the addition of the same ITPR protocol.

Electrolyte and Physiologic Monitoring

Blood samples were obtained at baseline, immediately following each injury, following 30 minutes of each device setting and following 15 minutes of recovery at 5 cm H_2O PEEP, prior to restarting the device at the next setting. 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, lactate, and base excess.

Serum and Tissue Analysis

Whole blood samples were obtained via the arterial catheter and centrifuged at 8000 rpm for 15 min in serum separator tubes. Lung tissue samples from left and right lower lobes were extracted via thoracotomy following euthanasia. All samples were stored at -80°C. Serum was then analyzed by multiplex enzyme-linked immunosorbent assay (ELISA), for pro-inflammatory cytokines including interleukin 1 beta (IL-1β), IL-6, IL-8, and tumor necrosis factor alpha (TNFα) (Quansys Biosciences, Logan, UT). Serum was also analyzed for porcine surfactant associated protein D (MyBioSource, Inc., San Diego, CA) as a potential marker of lung injury.

Histopathologic analysis

Following extraction after euthanasia, lung tissue was fixed in 4% paraformaldehyde. Sections were stained with hematoxylin and eosin and evaluated by three blinded, independent observers. Lung

injury was analyzed by a quantitative scoring system based on alveolar capillary congestion, infiltration of red blood cells and inflammatory cells into the airspace, alveolar wall thickness and hyaline membrane formation. [13]

Statistical Analysis

All statistical analysis was performed with SPSS 15.0 (IBM Corp., Armonk, NY). Continuous variables are represented as mean ± standard deviation or median (interquartile range), where appropriate based on value distribution. Continuous variables were compared using repeated measures ANOVA with contrasts (comparisons) determined prior to experimentation. A p value less than 0.05, with Sidak adjustment for inflated type I error, was considered significant. All vital signs, laboratory and neurologic monitoring values were compared between Sham (n=3), ITPR/Sham (n=3), Hem (n=5), ITPR/Hem (n=6), ALI/Hem (n=5), and ITPR/ALI/Hem (n=5). The average injury scores for each slide were compared between Sham (n=9), ITPR/Sham (n=9), Hem (n=24), ITPR/Hem (n=27), ALI/Hem (n=27), ITPR/ALI/Hem (n=27).

RESULTS

Cardiac function

Adequate shock was induced in the hemorrhage model, with the MAP being appropriately decreased in the Hem and ITPR/Hem group compared to Sham and ITPR/Sham, respectively, at all time points. (Figure 1a) The heart rate was consistently higher at all time points with the use of ITPR following hemorrhage in the ITPR/ALI/Hem group compared to the ALI/Hem group and higher in the ITPR/Hem group compared to Hem at -6, -9 and -12 cmH_2O during the recovery period. (Figure 1b) The cardiac output was higher in the ITPR/ALI/Hem group compared to ALI/Hem only post-ALI and at -9 cmH_2O. Cardiac output (CO) was similar at all settings in the ITPR/Hem group compared to the Hem group. (Figure 1c) The ITPR device did not have a significant effect on MAP regardless of injury, heart rate, or cardiac output.

Pulmonary function

Adequate hypoxia was induced in the lung injury model, with the PaO_2/FiO_2 ratio being appropriately decreased in ALI/Hem compared to Sham and Hem groups at all time points. (Figure 2a) The respiratory rate, adjusted to prevent respiratory acidosis, was higher in the ITPR/ALI/Hem group compared to ALI/Hem at -9 and -12 cmH_2O and higher in the ITPR/Hem group compared to Hem at -6, -9 and -12 cmH_2O. (Figure 2b) EELV was decreased in the ITPR/Sham and ITPR/Hem groups compared to Sham and Hem, respectively, at all time points. EELV was decreased in ITPR/ALI/Hem compared to ALI/Hem at all time points except -12 cmH_2O. In the ITPR/ALI/Hem group, EELV was decreased at all ITPR settings except -12 cmH_2O compared to the ALI/Hem group. (Figure 3a) Lung compliance was decreased in the ITPR/Sham and ITPR/Hem groups compared to Sham and Hem, respectively, at the -6, -9 and -12 cmH_2O settings. In the ITPR/ALI/Hem, the use of the ITPR device decreased lung compliance at -9 and -12 cmH_2O settings. (Figure 3b) The PaO_2/FiO_2 ratios were similar in the ITPR/Sham group compared to Sham, ITPR/Hem group compared to Hem and the ITPR/ALI/Hem group compared to ALI/Hem. (Figure 3c) The V_D/V_T was increased in the ALI/Hem group compared to both Hem and Sham groups, but there were no additional differences between the groups with the ITPR and their respective injury controls. (Figure 3d).

Effect of ITPR device on arterial blood gas, hemodynamic, pulmonary and cerebral parameters

There were no notable differences in temperature, ICP, cerebral perfusion pressure, central venous pressure, pulmonary capillary wedge pressure, tidal volumes, positive end expiratory pressure, pH, $PaCO_2$, bicarbonate, lactic acid, sodium, potassium, calcium, hematocrit or hemoglobin between any of the injury or ITPR groups when measured 15 minutes after cessation of each level of ITPR therapy.

IL-1b and IL-6 levels

Interleukin-1b (IL-1b) levels were increased compared to baseline starting values in the Hem (107.2±67.0 pg/mL vs. 289.4±132.7 pg/mL, p=0.03), ALI/Hem (1065.0±396.1 pg/mL vs. 2409.0±1090

pg/mL, p=0.03), and ITPR/ALI/Hem (1307.0±795.7 pg/mL vs. 6375.0±4373.0 pg/mL, p<0.01) groups. At baseline and after the -12 cmH_2O setting, the Hem group (107.2±67.0 pg/mL and 289.4±132.7 pg/mL, respectively) had lower levels compared to Sham (904.3±41.1 pg/mL, p<0.01 and 985.3±194.9 pg/mL, p<0.01, respectively). At baseline, serum IL-1b levels were lower in the ITPR group compared to Sham (731.3±63.0 pg/mL vs. 904.3±41.1 pg/mL, p=0.02). At baseline and -12 cmH_2O setting, IL-1b levels were higher in the ITPR/Hem group compared to Hem (1094.0±357.4 pg/mL vs 107.2±67.0 pg/mL, p<0.01 and 1473.0±915.2 pg/mL vs. 289.4±132.7 pg/mL, p=0.02, respectively). (Figure S1a) Interleukin-6 (IL-6) levels were increased over time in the ALI/Hem (951.4±421 pg/mL vs. 14,429.0±9,038 pg/mL, p<0.01) and ITPR/ALI/Hem groups (758.7±195.8 pg/mL vs. 30,344±17,194.0 pg/mL, p<0.01). At baseline, IL-6 levels were increased in the ITPR/Hem group compared to Hem (1024.0±704.9 pg/mL vs. 172.2±86.5 pg/mL, p=0.03). (Figure S1b) After the -12 cmH_2O setting, interleukin-8 (IL-8) levels were increased only in the ITPR/ALI/Hem group compared to ALI/Hem (45,908.0±24,883 pg/mL vs. 14,388.0±15,000 pg/mL, p=0.04). (Figure S1c) At baseline and after the -12 cmH_2O setting, TNF-α levels were decreased in the ITPR/ALI/Hem group compared to ALI/Hem (5,345.0±1,817.0 pg/mL vs. 9,827.0±4,285.0 pg/mL, p=0.04 and 7,427.0±2,539.0 pg/mL vs. 14,824.0±4,859 pg/mL, p<0.01, respectively). (Figure S1d) Following lung injury, TNF-α levels were also lower in the ITPR/ALI/Hem group compared to ALI/Hem (5244±2265 pg/mL vs. 13799±11810 pg/mL, p<0.01). There were no differences in porcine surfactant associated protein D between groups or over time.

Histopathologic analysis of lung injury

Lung injury was more severe in the ITPR/ALI/Hem group compared to ALI/Hem and ITPR alone group. The ALI/Hem group had worse lung injury compared to Hem and Sham. In addition, the ITPR/Hem group had worse lung injury compared to Hem and the ITPR alone group had worse lung injury compared to Sham. Interestingly the addition of ITPR (ITPR/Hem) or ALI (ALI/Hem) to hemorrhage alone (Hem) appeared to create a similar amount of additional lung injury (Figures 4a and b)

DISCUSSION

In this porcine polytrauma model, we utilized physiologic monitoring, serum biomarkers, neurologic monitoring, lung histology and arterial blood gas analysis to evaluate the effect of NEEP created by the ITPR device following hemorrhagic shock and acute lung injury. We established appropriateness of our injury model by demonstrating significant reduction in MAP following hemorrhage and PaO_2/FiO_2 ratio to be less than 250 following lung injury induced by surfactant washout. The increase over time of inflammatory cytokines, interleukin-1b and IL-6, demonstrated a systemic response to the combination of hemorrhage and lung injury. Importantly, pulmonary physiology and lung histopathology scoring demonstrated increased severity of lung injury with the use of ITPR device and in pigs that underwent acute lung injury.

Application of the ITPR device resulted in an overall increase in heart rate and cardiac output, but did not change the mean arterial pressure or other physiologic parameters measured by pulmonary artery catheter regardless of injury. A worsening of pulmonary function with use of the device was also observed. This was demonstrated by an increased respiratory rate, decreased EELV and pulmonary compliance following use of the ITPR device. The device also did not decrease ICP as previously described. [12] However, previous studies have utilized a space-occupying lesion (balloon catheter in the subdural space) to produce elevated ICP. Differences in the control of ICP in these models may explain the disparate findings as well as an inability to demonstrate elevated ICP in the setting of concomitant ongoing hypotension.

Our findings do not support previous literature that described the benefits of the ITPR device following hemorrhage. However, a few differences in the model designs may have some effect on these outcomes. [10,11] First, the mean arterial pressure was reduced to a near lethal level. In the previous study MAP was around 25 mmHg after a 55% bleed, while our model only reduced the MAP to 35 mmHg. Second, both previous studies used intra-aortic pressure rather than the femoral artery as in our protocol, which may differentiate small changes in MAP during device-induced changes in intrathoracic pressure. Third, the pigs in our study remained in hemorrhagic shock for two hours compared to the two minutes

prior to initiation of the ITPR device in the previous studies. Finally, the small number of pigs that survived the near lethal bleed in the previous study limits the definitive conclusions that can be drawn. [10] A study not utilizing the ITPR device but evaluating NEEP demonstrated an increase in mean arterial pressure, cardiac output, and short-term survival in pigs that underwent NEEP during ventilation. [14] Another study by Herff et al. demonstrated that reduction of positive end expiratory pressure was the most important ventilation strategy to improve hemodynamic stability. [15] In comparison to our study, both of these studies used a severe hemorrhage model (45 mL/kg of blood loss) and a shorter time span that may account for the absence of blood pressure benefit in our study. Additionally, the present polytrauma model may be a more severe injury that the device cannot overcome to induce a significant increase in mean arterial pressure.

There have been a few studies of utilizing the ITPR device in humans that deserve consideration and comparison to this porcine model. In a pilot study evaluating 10 intubated patients with elevated ICP despite ongoing standard medical therapies a significant decrease in ICP and increase in CPP with the use of the device was demonstrated. In contrast to our study, these patients had primary cerebral pathology and were not in concomitant hemorrhagic shock. The authors also discuss limiting the use to 10 minute applications due to the potential for adverse respiratory outcomes. While they did not observe any of these complications of the device, it is possible that longer application of the device would result in decreasing oxygenation. [16] Another study utilized the ITPR device for 2 consecutive hours in 5 patients with brain injury. Similar to the previous study, these patients did not have another concomitant injury such as lung injury or hemorrhage. This study demonstrated a significant increase in cerebral perfusion pressure by decreasing the ICP and increasing MAP. However, the 5 mmHg increase in MAP is unlikely to be clinically significant in a patient in hemorrhagic shock. [17] In addition, because both MAP and CPP decrease significantly with ongoing hemorrhage, it is not physiologically possible to demonstrate an improvement in ICP from the ITPR device, as it is already reduced by hemorrhagic shock. A final human study consisted of seven paired, young, healthy volunteers who were intubated, sedated, underwent

hemorrhagic shock and received either the ITPR device or placebo for 1 hour on separate days. This study also failed to show a significant improvement in MAP, but did show an increase in stroke volume. These studies show a decrease in ICP, but are similar to our findings that the ITPR device does not significantly increase MAP. The models also differ in that the first two do not consist of hemorrhagic shock and none of the models contain a lung injury. [18]

This study is the first to evaluate the ITPR device in a porcine lung injury model. We demonstrated that the ITPR device caused an increased respiratory rate, decreased EELV and worse PaO_2/FiO_2 ratio. We also demonstrate a more severe histopathologic lung injury in pigs in which the device was used compared to their respective injury controls. These novel findings suggest that the ITPR device may be harmful to the polytrauma patient with lung injury, similar to the induction of repeated atelectrauma. The current data support the ITPR device contraindication to utilization in the setting of acute lung injury. However, in emergency field care, acute lung injury may not be known or recognized. Pathologic lung injury severity was also worse in the pigs that received the ITPR device, but did not have acute lung injury and this is a novel finding. Additionally, we noted that at negative pressures > 6 cm H_2O, all pigs exhibited gasping. This was likely due to activation of the Hering-Breuer deflation reflex. [19] While gasping following cardiopulmonary resuscitation has been associated with improved outcomes in subjects through mechanisms that are not fully elucidated, presence of gasping may be a marker of improved cerebral circulation and respiratory drive. [20,21] In this study, the presence of gasping invalidated measures of EELV and compliance and as a consequence, all animals were chemically paralyzed to complete the study. While this is a limitation of our trial, it may also be an underappreciated but critical limitation of the device, as chemical paralysis in the setting of hemorrhagic shock would exacerbate post-traumatic hypotension.

The ITPR device relies on the physiologic observation that during spontaneous breathing, a decrease in intrathoracic pressure and increase in lung volume is associated with improved venous return and increased cardiac output. These salutary effects are the basis of intermittent mandatory ventilation

and airway pressure release ventilation. [22] Inspiration against a closed airway or resistance is the basis of the use of the ResQPod during cardiopulmonary resuscitation. Similarly, the ResQGuard device relies on spontaneous inspiration and decreased intrathoracic pressure at an increased lung volume [23] By contrast, the VPOD device used in this study creates negative intrathoracic pressure with a concomitant decrease in lung volume. These simple physiologic differences may explain these findings. It is also important to note that NEEP, the predicated modality for ITPR therapy, was introduced in the 1960's as an effort to reduce air-trapping in COPD. However, the net effect was earlier terminal airway collapse and worsening of gas-trapping, resulting in abandonment of NEEP as a respiratory adjunct. [24,25] Similarly, in this study, NEEP utilized in ITPR likely induced repetitive distal airway collapse, resulting in the worsened pulmonary function and lung injury observed.

In conclusion, our swine polytrauma model demonstrates appropriate physiologic and biomarker changes expected in hemorrhage and lung injury. While some physiologic changes are appreciated, the ITPR device did not demonstrate an appreciable effect on mean arterial pressure regardless of injury, heart rate or cardiac output. In addition, there may be a harmful pulmonary effect of the ITPR negative end expiratory pressure in the setting of acute lung injury.

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FIGURE LEGEND

Figure 1: a) Adequate shock was induced in the hemorrhage model, with the mean arterial pressure being decreased in the Hem and ITPR/Hem group compared to Sham and ITPR/Sham, respectively, at all time points. * = $p < 0.05$ b) The heart rate was consistently higher at all time points following hemorrhage in the ITPR/ALI/Hem group compared to the ALI/Hem group. It was also higher in the ITPR/Hem group compared to Hem at -6, -9 and -12 cmH_2O during the recovery period. * = $p < 0.05$ for ITPR/ALI/Hem vs ALI/Hem, # = $p < 0.05$ for ITPR/Hem vs Hem c) The cardiac output was higher in the ITPR/ALI/Hem group compared to ALI/Hem post-ALI and at -9 cmH_2O and higher in the ITPR/Hem group compared to Hem at -3 cmH_2O. * = $p < 0.05$ for ITPR/ALI/Hem vs ALI/Hem, # = $p < 0.05$ for ITPR/Hem vs Hem

Figure 2: a) Adequate hypoxia was induced in the lung injury model, with the PaO_2/FiO_2 ratio appropriately decreased in the ALI/Hem compared to Sham and Hem at all time points. * = $p < 0.05$ b) The respiratory rate (required to maintain pH) was higher in the ITPR/ALI/Hem group compared to ALI/Hem at -9 and -12 cmH_2O and higher in the ITPR/Hem group compared to Hem at -6, -9 and -12 cmH_2O. * = $p < 0.05$ for ITPR/ALI/Hem vs ALI/Hem, # = $p < 0.05$ for ITPR/Hem vs Hem

Figure 3: a) End expiratory lung volume (EELV) was decreased in the ITPR/Sham and ITPR/Hem groups compared to Sham and Hem, respectively, at all time points. It was decreased in ITPR/ALI/Hem compared to ALI/Hem at all time points except -12 cmH_2O. b) The lung compliance was decreased in the ITPR/Sham and ITPR/Hem groups compared to Sham and Hem, respectively, at the -6, -9 and -12 cmH_2O settings. In the ITPR/ALI/Hem, the use of the ITPR device decreased lung compliance at -9 and -12 cmH_2O settings. c) The PaO_2/FiO_2 ratios were similar in the ITPR/Sham group compared to Sham, ITPR/Hem group compared to Hem and the ITPR/ALI/Hem group compared to ALI/Hem. * = $p < 0.05$ for ITPR/ALI/Hem vs ALI/Hem, # = $p < 0.05$ for ITPR/Hem vs Hem, ^ = $p < 0.05$ for ITPR vs Sham d) The V_D/V_T was increased in the ALI/Hem group compared to Hem and Sham at all time points. * = $p < 0.05$

Figure 4: a) Lung scoring demonstrated worse injury severity in ITPR/ALI/Hem compared to ALI/Hem, ITPR/Hem and ITPR. ALI/Hem had worse injury severity compared to Hem and Sham. ITPR/Hem had worse injury severity compared to Hem. ITPR had worse injury severity compared to Sham. b) Representative hematoxylin and eosin staining of lung samples in each injury group.

Figure S1: a) Interleukin-1b (IL-1b) levels were increased from baseline in the Hem, ALI/Hem and ITPR/ALI/Hem groups. At baseline and at the -12 cmH_2O setting, the Hem group had lower levels compared to Sham. At baseline, IL-1b levels were lower in the ITPR group compared to Sham. At baseline and -12 cmH_2O setting, IL-1b levels were higher in the ITPR/Hem group compared to Hem. b) Interleukin-6 (IL-6) levels were increased from baseline in the ALI/Hem and ITPR/ALI/Hem groups. At baseline, IL-6 levels were increased in the ITPR/Hem group compared to Hem. c) At the -12 cmH_2O setting, interleukin-8 (IL-8) levels were increased in the ITPR/ALI/Hem group compared to ALI/Hem. d) At baseline and at the -12 cmH_2O setting, tumor necrosis factor alpha (TNF-α) levels were decreased in the ITPR/ALI/Hem group compared to ALI/Hem. * = $p < 0.05$ compared to respective injury model without ITPR device, § = $p < 0.05$ compared to Sham.

Figure 1

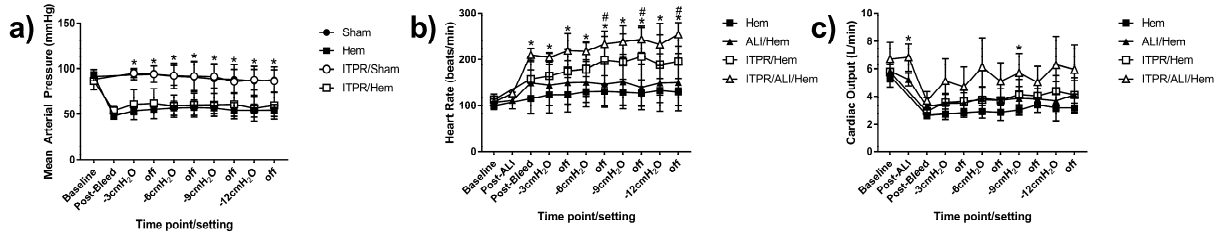

Figure 2

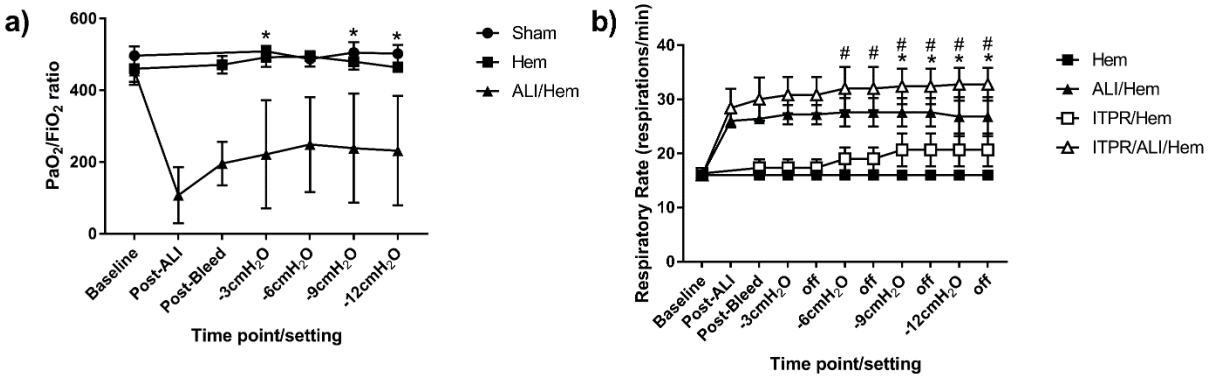

Figure 3

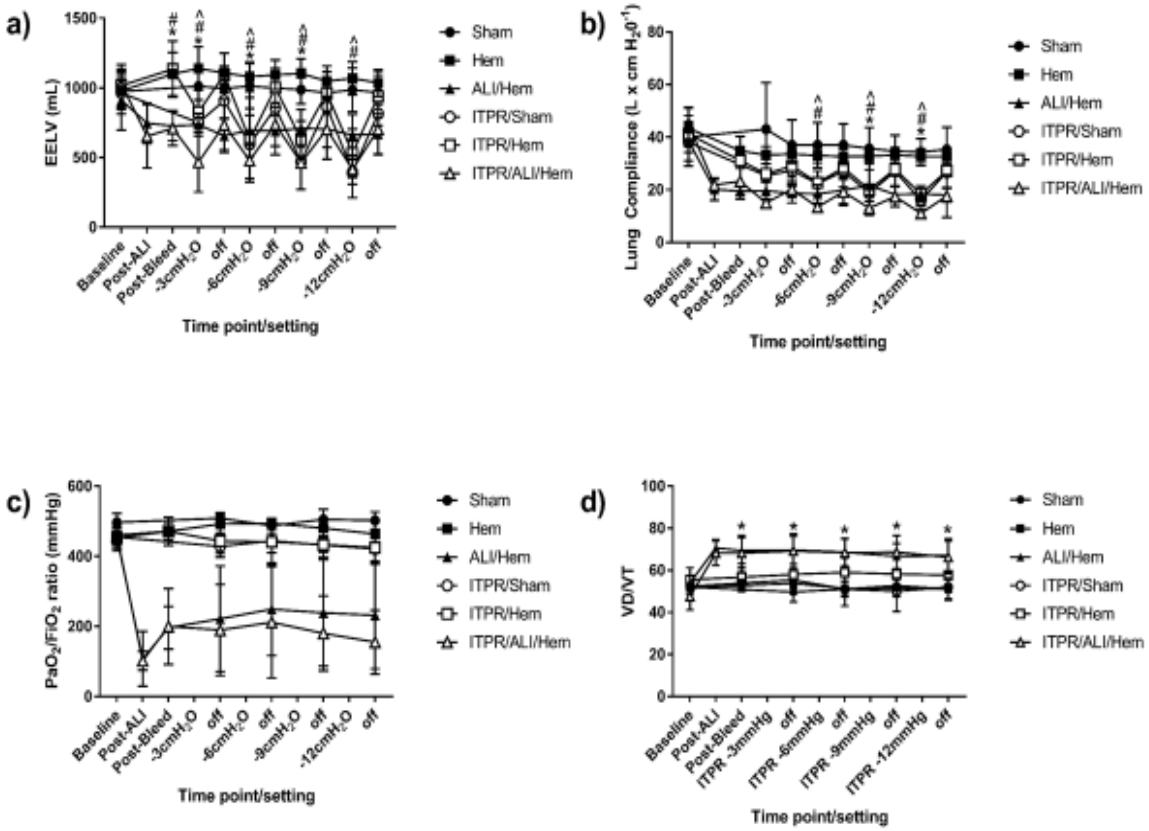

Figure 4

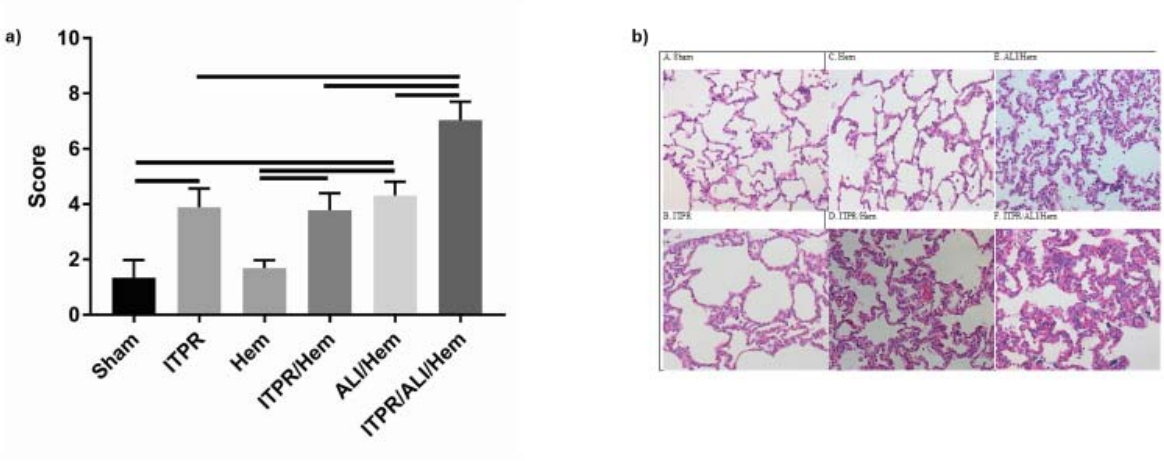

Figure S1