ABSTRACT

Background: In locations in which access to resuscitative therapy may be limited, treating polytraumatized patients presents a challenge. There is a pressing need for adjuncts that can be delivered in these settings. To assess these adjuncts, a model representative of this clinical scenario is necessary. We aimed to develop a hemorrhage and polytrauma model in the absence of fluid resuscitation. Materials and Methods: This study consisted of two parts: pulmonary contusion dose-finding (n = 6) and polytrauma with evaluation of varying hemorrhage volumes (n = 6). We applied three, six, or nine nonpenetrating captive bolt-guns discharged to the dose-finding group and obtained computed tomography (CT) images. We segmented images to assess contusion volumes. We subjected the second group to tibial fracture, pulmonary contusion, and controlled hemorrhage of 20%, 30%, or 40% and observed for 3 hours or until death. We used Kaplan-Meier analysis to assess survival. We also assessed hemodynamic and metabolic parameters. Results: Contusion volumes for three, six, and nine nonpenetrating captive bolt-guns were 24 ± 28, 50 ± 31, and 63 ± 77 cm³, respectively (p = .679). Animals receiving at least six discharges suffered concomitant parenchymal laceration, whereas one of two swine subjected to three discharges had lacerations. Mortality was 100% at 12 and 115 minutes in the 40% and 30% hemorrhage groups, respectively, and 50% at 3 hours in the 20% group. Conclusion: This study characterizes a titratable hemorrhage and polytrauma model in the absence of fluid resuscitation. This model can be useful in evaluating resuscitative adjuncts that can be delivered in areas remote to healthcare access.

Keywords: Polytrauma model; pulmonary contusion; controlled hemorrhage; tibial fracture; delayed medical care; prolonged casualty care; prolonged field care

Introduction

About 90% of potentially preventable combat deaths are the result of acute hemorrhage, with the majority occurring prior to admission to a medical facility.¹ Mechanical hemorrhage control has improved greatly; extremity tourniquets have reduced combat casualties 85%.² However, control via direct pressure is ineffective in the setting of noncompressible torso hemorrhage (NCTH), which accounts for two-thirds of combat hemorrhage related casualties.³,⁴

It is difficult to provide therapy to complex patients with NCTH or polytrauma, particularly in remote regions where access to resuscitation fluid and operative intervention are not immediately available.⁵ There is a pressing need for resuscitative adjuncts that can be utilized in patients with NCTH or polytrauma who have delayed access to appropriate medical care. To this end, we need an appropriate model to evaluate these potential resuscitative adjuncts. Though hemorrhage and polytrauma models exist, they importantly involve active fluid resuscitation within an acute timeframe.⁶,⁷ This simulates a level of access to care that does not reflect the context we hope to evaluate further.

In this study, the aim is to develop a reliable hemorrhage and polytrauma model in the absence of fluid resuscitation or other intervention. This model will include pulmonary contusion, tibial fracture, and controlled hemorrhage. The pulmonary contusion should be replicable and quantifiable. The ideal model would afford enough time for delivery of a resuscitative adjunct immediately after end of trauma but be lethal within a period of 2–3 hours in the absence of any intervention.

Methods

Study Design and Overview

The experiment was conducted at the University of Maryland School of Medicine, which is accredited by the American Association for Laboratory Animal Science. The Institutional Animal Care and Use Committee approved the protocol prior to the start of animal study. We used adult male Hanford miniature swine weighing between 60 and 80 kg. All swine were subjected to an acclimatization period of at least 48 hours under the care of licensed veterinary staff. We provided the animals free access to food and water until the night prior to surgery at which point we fasted them overnight.

We divided the study into two parts: pulmonary contusion dose-finding and delivery of polytrauma with evaluation of varying hemorrhage volumes. Both parts consisted of the same three phases: anesthesia and instrumentation, induction of trauma, and observation. We recorded hemodynamic indices continuously throughout the experiment. The laboratory purchased all equipment used to conduct the experiment. At the end of the protocol, we euthanized animals with potassium chloride (>2mmol/kg).

Anesthesia and Instrumentation

We sedated swine with Telazol (tiletamine and zolazepam for injection; 4~5mg/kg) and xylazine (1.5~2.2mg/kg) via intramuscular injection. We administered general anesthesia with isoflurane by facemask followed by orotracheal intubation.

*Correspondence to jonathan.morrison@som.umaryland.edu
**All authors are affiliated with the R Adams Cowley Shock Trauma Center, University of Maryland Medical System, Baltimore, MD.
We maintained general anesthesia with 2–2.5% isoflurane in 40–100% oxygen to maintain an oxygen saturation of >92%. We used volume-controlled mechanical ventilation with a respiratory rate of 10–15 breaths/min and a tidal volume of 12–15 mL/kg. We managed temperature with a warming blanket set to 38°C to prevent hypothermia.

We obtained percutaneous vascular access using a Seldinger technique to insert 7Fr sheaths (Terumo Medical Corporation, https://www.terumomedical.com/) in a carotid artery, bilateral femoral arteries, and an external jugular vein. We used OEC 9800 Plus fluoroscopy (General Electric, https://www.gehealthcare.com/) to facilitate placement of a pressure-volume (PV) loop catheter (Transonic Corporation, https://www.transonic.com/) in the left ventricle and solid-state pressure catheters (Transonic Corporation, https://www.transonic.com/) in the aorta and pulmonary artery. We placed a 25 Fr cannula in a femoral vein for execution of controlled hemorrhage.

We performed a mini-laparotomy for the purpose of placing a urinary cystostomy. We placed a chest tube in the right chest to prevent the potential development of a hemodynamically impactful pneumothorax in the process of creating a pulmonary contusion.

**Induction of Trauma**

**Pulmonary Contusion Dose-Finding**

We dedicated six swine to the pulmonary contusion dose-finding portion of the study. We created a pulmonary contusion in each of the six animals using a nonpenetrating captive bolt gun (Farmer Boy, https://farmerboyag.com/). When the nonpenetrating captive bolt gun was tested against a force sensor (Loadstar Sensors, https://www.loadstarsensors.com/), over an average of six discharges, the gun delivered a mean and standard deviation force of 21,322 ± 3,249 N. Prior to delivery of pulmonary contusion, we placed a right-sided 24 Fr chest tube to evacuate any pneumothorax that might be incurred during the chest injury. We put the chest tube to water seal throughout the experiment.

To deliver the pulmonary contusion, we secured the nonpenetrating captive bolt gun in place anterolaterally at the right chest using a custom-built frame. We inflated the lungs to a pressure of 30 cm H₂O for 20 seconds prior to each nonpenetrating captive bolt gun discharge to ensure apposition of the lung to the chest wall. We resumed normal ventilation between nonpenetrating captive bolt gun discharges. We divided the swine into three groups of two animals undergoing three, six, or nine nonpenetrating captive bolt gun discharges. We obtained noncontrast computed tomography (CT) images for each animal 1 hour after delivery of pulmonary contusion using a 16-slice OmniTom portable CT scanner (Neurologica, https://www.neurologica.com/).

**Delivery of Polytrauma**

We dedicated six swine to the delivery of polytrauma with evaluation of varying hemorrhage volumes part of the study. We subjected all animals to pulmonary contusion, tibial fracture, and controlled hemorrhage. We divided animals into three groups of two animals undergoing controlled hemorrhage of 20%, 30%, or 40% of their respective blood volumes. We estimated total blood volume assuming 66 mL of blood per kilogram for adult swine.⁸

We delivered pulmonary contusions as described above. Subsequently, we subjected swine to tibial fracture using a benchtop shop press (Northern Tool, https://www.northerntool.com/). Swine hind limbs were unrestrained for this procedure. We centered the tibia in the benchtop shop press, which we then used to apply pressure to the tibia until fracture was achieved. We used fluoroscopy to confirm fracture (Figure 1).

**Controlled hemorrhage** was accomplished using a peristaltic pump (Master Flex, https://www.masterflex.com/), which we connected to the 25 Fr cannula in the femoral vein. We hemorrhaged the animals over the course of 1 hour with two-thirds of the volume removed over the first 30 minutes and one-third of the volume removed over the second 30 minutes.

**Observation**

We observed animals for a maximum of 3 hours from the end of trauma. We did not complete any resuscitative interventions over the course of the observation period. We defined death as a sustained mean arterial pressure (MAP) of < 10mmHg for 1 minute and ECG activity incompatible with a spontaneous circulation. We euthanized swine that remained alive throughout the observation period using IV potassium.

**Data Collection**

We recorded animal weight for each experiment. We captured hemodynamic data including MAP, heart rate (HR), and cardiac output (CO) continuously using an integrated life science data acquisition system (ADInstruments, https://www.adinstruments.com/). We measured metabolic parameters including potassium (K) and lactate via arterial blood gas sampling performed at baseline, end of trauma, and death (Radiometer, https://www.radiometer.com/). We exported all data to Excel (Microsoft, https://www.microsoft.com/) for storage.

**Pulmonary Contusion Segmentation**

We segmented pulmonary contusions identified on CT using open-source software (Horos Project, www.horosproject.org). We segmented tissue where the HUs exceeded –351, using previously described and validated values from the literature.⁹ We obtained noncontrast computed tomography (CT) images of a representative pulmonary contusion (A) as well as an example of the segmented contusion volume (B). Figure 2 demonstrates a CT image of a representative pulmonary contusion.

**Experimental Outcomes**

Pulmonary contusion volumes and survival after polytrauma are the primary outcomes of this study. Hemodynamic and metabolic parameters including MAP, HR, CO, K, and lactate are the secondary outcomes.
Swine Polytrauma Model Without Fluid Resuscitation

Statistical Analysis
We performed data analysis using Prism (GraphPad, https://www.graphpad.com/) version 8. We present data using mean ± standard deviation. We assessed survival from end of trauma using Kaplan-Meier analysis. We used analysis of variance to compare hemodynamic and metabolic data among animals. We considered a $p$-value of less than .05 significant.

Results

Pulmonary Contusion Dose-Finding
We enrolled six animals for pulmonary contusion dose-finding. No animals were excluded from the study. The mean weight was 67 ± 4 kg. Pulmonary contusion volumes for three, six, and nine nonpenetrating captive bolt gun discharges were 24 ± 28, 50 ± 31, and 63 ± 77 cm$^3$, respectively ($p = .679$) (Figure 2C). All animals with six or greater nonpenetrating captive bolt gun applications also had a lacerative component to their pulmonary injury (Figure 2D). Only one of the two animals (50%) who received three nonpenetrating captive bolt gun applications had a lacerative component to their injury. We selected six nonpenetrating captive bolt gun discharges for the polytrauma model as it reliably generated a pulmonary contusion with a lacerative component that was most consistent between animals.

Evaluation of Polytrauma Model
We enrolled six animals for evaluation of the polytrauma model with varying hemorrhage volumes: 20%, 30%, and 40% of total blood volume. No animals were excluded. Mean weight was 69 ± 3 kg. Baseline hemodynamic and metabolic data was comparable between animals and is presented in Table 1.

Survival
Kaplan-Meier analysis demonstrated 100% mortality at 12 and 115 minutes in the 40% and 30% hemorrhage groups, respectively (Figure 3). There was 50% mortality at 3 hours in the 20% hemorrhage group.

Hemodynamic Data
We compared hemodynamic data during trauma and hemorrhage, as well as during the observation period across the three groups (Figure 4). During trauma and hemorrhage, the 20%, 30%, and 40% hemorrhage groups had a mean heart rate of 119 ± 71, 169 ± 31, and 149 ± 59 bpm ($p = .140$), respectively. The mean MAPs were significantly different at 66 ± 20, 51 ± 2, and 40 ± 4 mmHg ($p = .040$), as were the mean cardiac outputs: 3.9 ± 0.9, 1.9 ± 0.1, and 1.4 ± 0.5 L/min ($p = .016$).

During the observation period, the 20%, 30%, and 40% hemorrhage groups had a mean heart rate of 148 ± 39, 121 ± 31, and 31 ± 22 ($p = .020$), respectively. The mean MAPs were 51 ± 32, 24 ± 6, and 8 ± 1 mmHg ($p = .030$), and the mean cardiac outputs were 5.2 ± 4.0, 1.1 ± 0.2, and 0.1 ± 0.2 L/min ($p = .080$).
**Metabolic Data**

We compared metabolic data trauma and hemorrhage as well as during the observation period across the three groups (Figure 5). During trauma and hemorrhage the 20%, 30%, and 40% hemorrhage groups had a mean $K$ of $6.7 \pm 3.3$, $7.7 \pm 1.7$, and $7.2 \pm 2.4$ mmol/L ($p = .737$). The mean lactates were $3.9 \pm 3.1$, $3.5 \pm 0.1$, and $3.8 \pm 1.7$ mmol/L ($p = .888$).

At death, the 20%, 30%, and 40% hemorrhage groups had a mean $K$ of $7.3 \pm 2.5$, $10.4 \pm 0.7$, and $7.2 \pm 2.4$ ($p = .340$). The mean lactates were $4.3 \pm 3.8$, $5.9 \pm 1.5$, and $3.8 \pm 1.7$ ($p = .700$).

**Discussion**

We developed a model of hemorrhage and polytrauma in the absence of fluid resuscitation that incorporates three components: tibial fracture, pulmonary contusion, and controlled hemorrhage. The group that was subjected to the 30% hemorrhage demonstrated optimal survival time characteristics given our aims. However, importantly, two components of this injury model are titratable (pulmonary contusion and controlled hemorrhage). This is reflected by the pulmonary contusion dose-finding results with quantified contusion volumes, as well as the stepwise progression of the mortality, hemodynamic, and metabolic data. Thus, the model can be adjusted as needed to suit an investigator’s needs.

Our summation model was designed to provide an Injury Severity Score (ISS) > 16 across all groups to be consistent with polytrauma as previously defined.10,11 This included extremity fracture, which contributed 9 points, and pulmonary contusion with laceration, which contributed 16 points, for an overall ISS of 25. The ISS and the Trauma Injury Severity Scale (TRISS), however, fail to predict the overall course of morbidity in these injury patterns as a result of their inability to capture physiologic, metabolic, and kinetic loading parameters.11 Previous murine models have demonstrated that the pattern of injury as opposed to the severity of the injury is perhaps more important with respect to thresholds of mortality.12

Our model is further reflective of this. The pulmonary contusion dose finding component, despite being limited to one system, produced variable results suggesting that injury severity correlated poorly with the measured reactive force. This is likely due to the viscoelastic properties of the porcine thoracic cavity and the necessary viscous criteria to produce the desired results.11 It has previously been suggested that this plastic deformation of the rib cage alone can account for the linear relationship of the Abbreviated Injury Scale (AIS) that comprises the ISS but does not describe the underlying anatomic tension that can lead to larger inflammatory and physiologic derangements.10,12 An injury described as an AIS of 3 as compared to an AIS 4 is reflective only of a 10% change in compression depth in a linear relationship, but does not necessarily reflect what this viscoelastic parameter does to the underlying parenchyma as a whole.13,14 This is such that lower velocity injuries repeated over time with sufficient strain patterns would be capable of producing equivocal tissue destruction and similar outcomes, despite different biomechanical patterns as in our model.

Further, with the addition of an extremity injury and subsequent hemorrhagic shock, the effects on mortality become even more clearly independent of ISS and that predicted by TRISS. As has been previously described, hemorrhagic shock, un-resuscitated as in our model, leads to a significant systemic inflammatory cascade that compounds multisystem organ dysfunction.10,17 This is through some combination of, as of yet, incompletely understood mediators, endothelial dysfunction, and the acidosis and coagulopathy that portend a worse outcome in hemorrhagic shock.11 Concomitantly, we found subsequently worse hemodynamic and metabolic disorders as hemorrhage was allowed to continue unabated, leading to subsequent cardiac collapse. This was also manifest with respect to loss of cardiac output markers of left-ventricular function. The same molecular inflammatory mediators of loss of
vascular integrity have also been noted to subsequently cause remote changes in cardiac gap junctions, subcellular apoptosis, and loss of function. While it could certainly be argued that there was potential blunt cardiac injury, the initial evidence of cardiac responsiveness to hemorrhage in accordance with loss of preload as manifest by increases in systemic vascular resistance and contractility argues against this premise. We also found increased extracellular potassium, a normally intracellular cation, as hemorrhage proceeded, suggesting a surrogate for ongoing apoptosis.

This study is limited by the small sample size, however the results do still demonstrate differential survival characteristics between groups as well as distinctive hemodynamic and metabolic patterns. We also did not include a nonhemorrhage comparator group to this particular model. However, the use of multiple injury patterns alone has already been demonstrated to be an independent predictor of poor outcomes, regardless of ISS. In particular, it has been shown with the use of long bone fractures, both in humans and in swine.

Conclusion

This model development study used advanced technology to characterize a polytrauma and hemorrhagic shock model in the absence of fluid resuscitation and the components therein. The data suggested the optimal model in this study would include a 30% controlled hemorrhage, a pulmonary contusion created using six nonpenetrating captive bolt gun discharges, and a tibial fracture. However, it is important to note the titratable nature of the model, allowing for adjustments to be made by individual researchers to suit their individual needs.

Quantifying the pulmonary contusion and demonstrating the differential survival of the various hemorrhage volumes contribute to the titratability and reproducibility of the model. This model allows for the capture of multiple hemodynamic and metabolic parameters which can help to expand the AIS for various organ systems, independent of variable loading constraints. This can help to delineate additional physiologic and kinematic thresholds for further multivariate analysis and subsequent therapeutic trials.

Disclosures

The authors have indicated they have no financial relationships relevant to this article to disclose.

Conflicts of Interest

None.

Author Contributions

HA, NP, JE, MJR, and JJM conceived the study concept. HA, NP, JE, MJR, JD, and DP conducted experiments and collected the data. NE and JD analyzed the data. HA and DP wrote the manuscript. JJM was primarily responsible for critical revisions. All authors read and approved the final manuscript.

References

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