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Valproic Acid Improves Survival and Decreases Resuscitation Requirements In a Swine Model of Prolonged Damage Control Resuscitation

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Abstract

Background: Although damage control resuscitation (DCR) is routinely performed for short durations, prolonged DCR may be required in military conflicts as a component of prolonged field care. Valproic acid (VPA) has been shown to have beneficial properties in lethal hemorrhage/trauma models. We sought to investigate whether the addition of a single dose of VPA to a 72-hour prolonged DCR protocol would improve clinical outcomes.

Methods: Fifteen Yorkshire swine (40–45 kg) were subjected to lethal (50% estimated total blood volume) hemorrhagic shock (HS) and randomized to 3 groups: 1) HS; 2) HS-DCR; 3) HS-DCR-VPA (150 mg/kg over 3 hours) (n=5/cohort). In groups assigned to receive DCR, tactical combat casualty care (TCCC) guidelines were applied (1 hour into the shock period), targeting a systolic blood pressure (SBP) of 80 mm Hg. At 72 hours, surviving animals were given transfusion of packed red blood cells, simulating evacuation to higher echelons of care. Survival rates, physiologic parameters, resuscitative fluid requirements, and laboratory profiles were used to compare the clinical outcomes.

Results: This model was 100% lethal in the untreated animals. DCR improved survival to 20%, although this was not statistically significant. Addition of VPA to DCR significantly improved survival to 80% ($p < 0.01$). VPA-treated animals also had significantly ($p < 0.05$) higher SBPs, lower fluid resuscitation requirements, higher hemoglobin levels, and lower creatinine and potassium levels.

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Author Contributions

Conception and design – AMW, HBA. Data acquisition – AMW, UFB, BEB, NJG, KC, JZ, ISD, CV, RK; Data interpretation – AMW, UFB, BEB, NJG, KC, JZ, ISD, RMR, YL, HBA; Manuscript preparation – AMW, UFB, BEB, ISD; Critical revisions – all authors.

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Conclusions: VPA administration improves survival, decreases resuscitation requirements, and improves hemodynamic and laboratory parameters when added to prolonged DCR in a lethal hemorrhage model.

Keywords

prolonged damage control resuscitation; prolonged field care; valproic acid; pro-survival strategies; hemorrhagic shock

Background

In the United States (U.S.), trauma is responsible for 59% of deaths between the age of 1 and 44.(1, 2) Moreover, five million people worldwide die each year secondary to injuries.(3) In the majority of cases, hemorrhage is the leading cause of death. Although most of these deaths occur within minutes of injury, nearly a third occur during the pre-hospital phase. These deaths may be preventable with improved treatment strategies.(4) The potential for reducing mortality rates is even greater in military trauma patients. In austere environments, 87% of deaths occur prior to arrival at medical centers.(5) Of those deaths, nearly 25% are considered potentially survivable, and nearly all of these involve hemorrhage.(5) For patients who survive transfer to medical facilities, 85% die within an hour of arrival.(6) As such, development of strategies to prolong survival in the pre-hospital setting and early on in the hospital course remains a priority.

Current strategies to prolong survival following hemorrhage involve damage control resuscitation (DCR), which has gained momentum in trauma care in recent years. The principles of DCR prioritize blood administration while minimizing crystalloid use.(7) Fresh whole blood (FWB) or blood product components are administered early as they have been shown to confer a survival advantage following hemorrhage.(8, 9) However, significant logistical constraints, including limited resources in the battlefield, often limit the administration of blood products in the pre-hospital environment. Although crystalloids are inexpensive and have an immediate impact on blood pressure, large volumes are often required (10) and excessive use can cause hemodilution,(11, 12) acidosis,(13) pulmonary edema,(14) and impaired immune functions.(15) These resuscitative strategies are acceptable for short-term use. However, the duration of field care is highly variable and can sometimes extend up to several days.(16). Traditionally, improvements in the trauma care system have focused on shortening the pre-hospital evacuation times. However, in future conflicts or mass trauma events, it is anticipated that the initial evacuation time, and thus initial surgical hemorrhage and contamination control, may be delayed for hours or days.(17) It is also believed that the conventional DCR strategies may not be adequate to meet the challenges posed by this period of “Prolonged Field Care” (PFC). Therefore, the U.S. Military is funding the development of novel strategies that would allow the medics to deliver extended support to the injured (up to 72 hours), while waiting for evacuation to higher levels of care.(18)

In recent years, adjunctive pharmacological agents have shown promise in improving outcomes in trauma. Valproic acid (VPA), a histone deacetylase inhibitor, has been found to

confer cytoprotection and promote a pro-survival phenotype through rapid and reversible acetylation of histone and non-histone proteins.(19) In large animal models of lethal hemorrhage,(20) polytrauma,(21, 22) septic shock,(23) ischemia-reperfusion,(24, 25) and TBI,(26–28) VPA has been shown to improve survival and mitigate organ dysfunction. In shortterm models of lethal hemorrhage and polytrauma, single-dose VPA treatment has been shown to improve survival even in the absence of blood product administration.(22) The extent of the impact of VPA in a PFC scenario, however, remains unknown.

In this study, we sought to investigate whether the addition of a single dose of VPA would improve outcomes in a swine model of lethal hemorrhagic shock (HS) in a simulated PFC environment. We hypothesized that VPA administration would improve survival, hemodynamics, and laboratory parameters compared to the conventional prolonged DCR.

Methods

The protocol for this study was reviewed by the University of Michigan Institutional Animal Care and Use Committee. Experiments were performed in compliance with all regulations regarding animal welfare and research. Strict aseptic technique was employed for all surgical procedures. The experimental timeline is represented in Figure 1.

Animal Selection and Acclimatization

Female Yorkshire swine (5–6 months, 40–45 kg; Michigan State University, East Lansing, MI) were used for this study. Animals underwent a minimum 5-day acclimation period before undergoing the study.

Anesthesia and Perioperative Drug Administration

Animals were induced using Telazol® (0.5mg/kg intramuscular injection; Pfizer, New York, NY) and inhaled isoflurane (2–4%) prior to endotracheal intubation (7.0mm tube). Following intubation, isoflurane (1–3%) was used for maintenance anesthesia. Isoflurane was titrated to achieve an appropriate depth of anesthesia. Peripheral intravenous (PIV) catheters (18–22 G) were placed in bilateral ear veins. A transdermal fentanyl patch (75*u*g/hr) was applied to the animal and left in place for the duration of the study. Prophylactic antibiotics (cefazolin, 1g 8-hourly) were administered perioperatively, and continued for 24 hours postoperatively.

Instrumentation and Monitoring

Using cut-down technique, the right femoral artery was isolated and a 5F 11cm catheter (Super Sheath, Boston Scientific Corporation, Marlborough, MA) was placed to facilitate hemorrhage. Using ultrasound-guidance, an 8F 11cm insertion sheath (Arrow, Libertyville, IL) was placed into the right external jugular vein, which was used to float a pulmonary artery catheter. Using cut-down technique, the left external jugular vein and carotid artery were isolated. A 5F 11-cm catheter (Super Sheath) was placed into the left carotid artery for blood pressure monitoring and blood collection, and an 8F 11-cm catheter (Super Sheath) was placed into the left external jugular vein for fluid administration. Both of these lines were tunneled posteriorly to the left lateral neck. A mini-laparotomy was performed for

placement of a suprapubic cystostomy tube into the bladder, which was temporarily secured with a purse-string suture.

Lethal Hemorrhage

Animals underwent removal of 50% of the estimated total blood volume (ETBV (mL) = weight (g) x 0.06 + 0.77) via the femoral artery catheter (½ over 7 minutes; remaining ½ over 13 minutes) using a Masterflex pump (Cole Palmer, Vernon Hills, IL). Blood was collected in standard blood collection bags (CPDA, AS-5; Terumo, Ann Arbor, MI). Whole blood was centrifuged and separated into packed red blood cells (pRBCs) and stored for transfusion at the 72-hour time point.

Shock, Damage Control Resuscitation, and Treatment

To simulate a worst-case scenario with delayed medic response time, animals were left in shock for 1 hour. After the shock period, animals underwent block randomization and assignment to 3 groups: 1) HS (control), 2) HS-DCR, and 3) HS-DCR-VPA (n=5/cohort). Animals in the HS group did not receive any fluid resuscitation. Groups assigned to receive DCR were given an initial 250mL bolus of 6% hetastarch (simulating limited colloid supplies) followed by subsequent 250mL boluses of lactated Ringer's (LR) solution administered as needed to sustain a systolic blood pressure (SBP) of at least 80 mm Hg over the 72-hour period, as recommend by the Tactical Combat Casualty Care (TCCC) guidelines.(7) Fluid boluses were infused over a 15-minute period using the Masterflex pump. Animals randomized to receive VPA treatment were administered a single dose (150mg/kg over 3 hours) at the start of the DCR. The duration of PFC (72 hours) was determined by the funding agency.

72-Hour Prolonged Field Care

Animals underwent continuous monitoring and received ongoing DCR over the 72-hour PFC period as needed. To reflect the logistical constraints of far-forward military settings, a maximum total volume of LR resuscitation was set at 100ml/kg (4000–4500mL). Electrolyte disturbances, including hypoglycemia and hyperkalemia, were not treated in accordance with the limitations of an austere environment.

An attempt was made to wean all the animals from mechanical ventilation as soon as they met the extubation criteria (Table 1). Animals that met these criteria had invasive monitoring discontinued; however, the tunneled 5F and 8F arterial and venous catheters were left in place. The arterial catheter was used for blood pressure monitoring (every 2 hours) and arterial blood gas collection. The venous catheter was used for fluid administration according to the DCR protocol. All incisions were closed in multiple layers with absorbable sutures and skin glue. Administration of isoflurane was discontinued, and animals were then weaned from mechanical ventilation. After extubation, these animals were returned to their housing area. Animals that failed to meet the criteria for extubation were kept under anesthesia, and mechanical ventilatory support was continued.

Animals that survived the full 72-hour PFC period were given a transfusion of autologous pRBCs over 1 hour. This simulated a delay of 72 hours prior to transfer to a higher echelon

of care where blood products were available. Animals were also given additional resuscitation fluids as needed to correct their laboratory parameters to within 10% of baseline. The study ended at 76 hours post-hemorrhage.

Intraoperative and Post-Injury Monitoring

The following physiological parameters were monitored and recorded continuously during mechanical ventilation: heart rate (HR), SBP, diastolic blood pressure (DBP), mean arterial pressure (MAP), central venous pressure (CVP), cardiac output (CO), core body temperature, and pulse oxygenation. These parameters were measured every 15 minutes. After extubation, heart rate, SBP, DBP, and MAP were recorded every 2 hours.

Blood Collection

Arterial blood gases (ABGs; Nova Biochemical, Waltham, MA) and whole blood samples were collected at baseline (BL), post-shock (PS; start of DCR and VPA administration), post-treatment (PT; end of VPA infusion), and 8 hours post-injury. Samples were also collected on post-injury day (PID) 1 (24 hours), 2 (48 hours), 3 (72 hours), and at the end (E, 76 hours) of the experiment. Blood samples were collected at the time of death for animals that died prior to the end of the experiment. ABGs were also collected at the previously mentioned intervals during the experiment. Blood samples were withdrawn into vacuum-sealed tubes, centrifuged for isolation of serum and plasma, and flash frozen for future analysis. Blood samples were included in the calculation of the hemorrhage volume. Blood samples were also sent for biochemical analysis including creatinine, creatine phosphokinase (CPK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TBILI).

Tissue Collection

After completion of the experiment (76 hours), animals were euthanized (Euthasol Euthanasia Solution, 0.1ml/kg body weight) prior to organ harvest. Tissue samples (brain, heart, lungs, liver, kidney, spleen, and bowel) were collected and flash frozen and fixed, respectively. Samples allocated for fixation were immersed in 4% paraformaldehyde for 24 hours, and then preserved in 70% ethanol for subsequent analysis. The same tissue collection methods were applied for animals that died early.

Endpoints and Statistical Analyses

The primary endpoints were survival and the fluid resuscitation volumes. Secondary endpoints included hemodynamic, laboratory, and biochemical parameters. Pilot experiments, which were not included in this study, were used to conduct a power analysis with a biostatistician. Using survival and fluid resuscitation volumes as the primary endpoints, this study was powered to detect a minimum difference of 60% between the HS-DCR and HS-DCR-VPA groups with an alpha of 0.05 and power of 80%.

All statistical analyses were performed using GraphPad Prism v6.00 (GraphPad Software, San Diego, CA). Survival and extubation rates were compared using Kaplan-Meier method with log-rank testing. One-way analysis of variance (ANOVA) with Tukey post-hoc testing was used to compare different variable between the three groups, whereas a Student's *t*-test

was used when only two groups were being compared at discrete time points. The Brown-Forsythe test was used to check for differences in variance between the groups. Data are expressed as mean ± standard deviation (SD), unless specified otherwise. Statistical significance was defined as $p < 0.05$.

Results

Survival

The Kaplan Meier curves for survival among the three groups are shown in Figure 2. This model was 100% lethal in the HS group. DCR improved survival to 20%, although this was not statistically significant. However, the addition of VPA to DCR significantly improved ($p < 0.01$) 76-hour survival to 80%.

Extubation

Significant differences in timing and rates of extubation were observed between the groups. VPA-treated animals had a significantly higher rate of extubation compared to the other groups (extubation rate, % – HS: 0%; HS-DCR: 40%; HS-DCR-VPA:100%;$p < 0.01$) (Figure 3). The animals in the HS-DCR group required mechanical ventilation for longer periods compared to the animals in the HS-DCR-VPA group.

Hemodynamic and Physiologic Parameters

No significant differences were seen in the baseline hemodynamics and physiological variables among the different groups. All animals experienced tachycardia and a predictable decrease in the SBP, CO, and CVP immediately following hemorrhage (Figure 4).

Following the initiation of DCR and VPA treatment in the respective groups, significant differences were noted in the SBP between the groups. In the HS-DCR-VPA group, SBP was significantly elevated ($p < 0.05$) at several discrete time points compared to the HS-DCR group from the onset of DCR to PID1 (Figure 4A). The animals that survived beyond PID1 had similar SBP readings for the remainder of the study, regardless of the group allocation. Several trends (although not statistically significant) were also noted in other hemodynamic and physiologic responses between the groups. HR was elevated in the HS-DCR-VPA group as compared to the HS-DCR group from the onset of DCR to PID1 (Figure 4B). However, the animal in the HS-DCR group that survived to the end of the experiment (n=1) had a higher HR from PID1 to PID3 as compared to the surviving animals in the HS-DCR-VPA group (n=4). This difference dissipated following blood transfusion on PID3. Following onset of DCR, CVP was elevated in the HS-DCR animals compared to the HS-DCR-VPA group (Figure 4C). Similarly, CO was elevated in the HS-DCR-VPA group compared to the HS-DCR group after onset of DCR (Figure 4D).

Resuscitation Requirements

The HS-DCR-VPA group required significantly less LR resuscitation compared to the HS-DCR group to maintain the target blood pressure (LR fluid resuscitation, ml/kg – HS-DCR-VPA: 31.8 ± 7.6 ml/kg; HS-DCR: 91.8 ± 18.3 ml/kg; $p < 0.001$) (Figure 5).

Laboratory Parameters

No differences were seen in the pH, partial pressure of CO_2, partial pressure of oxygen, sodium, potassium, glucose, hemoglobin, or lactate levels at the BL, PS, or PT time points among the three groups (Table 2). However, significant differences were noted in several parameters at the 8-hour time point. VPA-treated animals were noted to have significantly higher hemoglobin levels than animals in the HS-DCR group (Hb, g/dl – HS-DCR: 5.9 ± 1.1; HS-DCR-VPA: 8.2 ± 0.9; *p = 0.04).* VPA-treated animals also had significantly lower potassium levels (K, mM – HS-DCR: 6.5 ± 0.9; HS-DCR-VPA: 4.7 ± 0.7; *p = 0.03*). There was also a trend toward increased lactate levels in the VPA-treated group compared to the HS-DCR group, although this was not statistically significant. No other significant differences were observed between the groups.

Biochemical Parameters

No significant differences were noted in creatinine levels at the BL, PS, and PT time points (Supplemental Table). However, VPA-treated animals had significantly lower creatinine levels at 8 hours post injury compared to animals in the HS-DCR group (creatinine, mg/dL – HS-DCR: 2.98 ± 2.15; HS-DCR-VPA: 2.15 ± 0.25*;p = 0.02*). Similarly, there were no differences in CPK and AST levels at BL, PS, and PT time points between groups. However, there was a trend toward decreased CPK and AST levels in VPA-treated animals compared to those in the HS-DCR group at 8 hours post injury (CPK, mg/dL – HS-DCR: 4588 ± 2390; HS-DCR-VPA: 1509 ± 530; p = 0.12) (AST, U/L – HS-DCR: 584 ± 749; HS-DCR-VPA: 85.7 ± 25.8; p = 0.16). No significant differences were noted in ALT, ALP, and TBILI levels among groups at any time point.

Discussion

In this study, we evaluated whether the addition of VPA to the prolonged DCR protocol would improve outcomes in a swine model of lethal HS and PFC. We found that the addition of single-dose of VPA (150 mg/kg) significantly improved survival and decreased the resuscitation fluid requirements compared to both the no treatment and conventional DCR groups. We also found that VPA treatment improved laboratory and biochemical parameters. As providing care in austere environments and far-forward settings is often complicated by delays in evacuation and logistical constraints, VPA administration represents a potential adjunct to DCR that could serve as a “bridge” to definitive care.

In recent years, optimizing PFC has become a priority for the U.S. Special Operations Forces (SOF) medical experts.(17) PFC includes provision of care beyond the standard timelines to military forces deployed in remote and austere locations, and may involve staged care with first responders and evacuation procedures.(17) As the military continues to encounter novel and challenging combat casualty care scenarios, a gap remains in addressing the prolonged care needs for the injured soldiers. Not surprisingly, the military is focusing on enhancing its capabilities to deliver more effective PFC. Keeping an injured soldier alive for a prolonged period of hours to days with limited resources is a complex task. Although immediate hemorrhage control remains the key initial priority, these patients

also require appropriate resuscitation to maintain acceptable organ functions until the time of evacuation.

The concept of DCR has gained favor as a major tenet of PFC. DCR prioritizes early blood product administration, avoidance of crystalloids, and prevention of acidosis, hypothermia, and coagulopathy.(29, 30) TCCC guidelines are founded on the principles of DCR. These guidelines recommend that patients should be resuscitated until a palpable radial pulse, a SBP of 80–90 mm Hg, or normal mental status are achieved. Blood product administration, involving FWB or plasma, pRBCs, and platelets in a 1:1:1 ratio, is prioritized.(31, 32) When these products are not available, dried plasma should be administered, if feasible. 6% hetastarch and LR are recommended for resuscitation when blood products are not available. (31, 32) The PFC Working Group (PFC WG) recommends a similar approach where FWB is the fluid of choice for patients in HS.(33, 34) Although crystalloid and colloid choices have been debated, the PFC WG states that “the best fluid, however, is the one you have available.”(33) The duration of PFC is highly variable and has been documented to extend up to 120 hours.(16) Therefore significant logistical constraints may limit FWB or blood product components availability. Furthermore, multiple casualties can rapidly deplete limited resources. As such, optimization of DCR and development of life-sustaining adjunctive strategies has become a priority.

In recent years, VPA, a histone deacetylase inhibitor, has emerged as a promising pharmacologic treatment for hemorrhage and various traumatic injuries. VPA administration produces epigenetic changes by promoting acetylation of histone and non-histone proteins, reversibly altering chromatin conformation and promoting greater accessibility to transcription factors.(19) These molecular changes promote up-and downregulation of gene expression. In both small and large animal models of HS, VPA administration has been shown to significantly improve survival.(20–22, 25, 35, 36) VPA has even been shown to significantly improve early survival in severe polytrauma and HS as an independent treatment, comparable to transfusion with FWB.(22) These previous studies had focused primarily on improving the early survival (12 hours), but given the increasing importance of PFC and prolonged DCR in the military, we designed the current study to test VPA in the setting of delayed (72 hours) evacuation. This is the first study to demonstrate that VPA treatment significantly improves survival in the setting of prolonged DCR for up to 72 hours. Furthermore, this was achieved with a much lower dose of VPA (150 mg/kg) compared to what was used in the initial studies (300–400 mg/kg). Additional dose optimization studies are currently ongoing, and preliminary data appear to validate these findings.

With the logistical constraints of prehospital settings, maximizing the use of all the resources, including resuscitation fluids, becomes important. In this study, we found that VPA-treated animals required significantly lower volumes of LR resuscitation compared to the HS-DCR group. On average, VPA-treated animals required only 1/3rd of the fluid volumes to maintain the target SBP of 80 mmHg. VPA-treated animals also maintained a higher average HR and CO during the early DCR, which may be responsible for the significantly higher average SBP in this study. Other studies have demonstrated similar findings. For example, in a canine model of HS (42% hemorrhage), VPA-treated (100 mg/kg) animals had significantly higher MAPs compared to controls after being given

matched fluid resuscitation at different time points over a 72-hour period.(35) In a swine model of hemorrhage (35% total blood volume) followed by truncal ischemia-reperfusion injury, VPA-treated (400 mg/kg) animals had significantly decreased crystalloid resuscitation and epinephrine requirements when using objective resuscitation thresholds (MAP of 40 mmHg and CVP of 4 mmHg).(25) As VPA administration may help to increase SBP and decrease overall crystalloid administration requirements, it may be a high-impact strategy in helping conserve limited resources and allowing treatment of a greater number of patients in logistically-constrained environments.

We also found that VPA treatment was associated with favorable laboratory and biochemical profiles. VPA-treated animals had a significantly higher hemoglobin levels compared to animals in the HS-DCR group. This most likely reflects the fact that the animals treated with conventional DCR had worse hemodilution due to higher volume crystalloid resuscitation. The higher hemoglobin levels in VPA-treated animals may also have blunted the tachycardia (in response to anemia) after the PID1. We also observed that VPA-treated animals had significantly lower creatinine levels and less evidence of acute kidney injury compared to the HS-DCR group. This is in line with previous reports.(35, 37) In both small and large animal studies, VPA treatment has been shown to attenuate acute kidney injury and prevent apoptosis of kidney cells by upregulating phosphorylation of pro-survival protein Akt and decreasing the expression of pro-apoptotic BAD protein.(35, 37) Lastly, VPA-treated animals had significantly lower serum potassium levels, which is likely to be multifactorial in nature. VPA treatment provides cytoprotection, which could attenuate the migration of potassium from the cells into the circulation. Animals in the HS-DCR group also had a lower SBP compared to VPA-treated animals, which may have resulted in decreased perfusion, increased tissue hypoxia and cellular injury, and subsequent hyperkalemia, a well-known finding associated with severe hemorrhage.(38, 39) However, these findings may also be reflective of VPA's ability to provide renal protection, preventing hyperkalemia associated with acute kidney injury. Further studies to explore these findings are ongoing.

The mechanisms behind VPA's beneficial effects in HS have been investigated in recent years. VPA treatment has been shown to induce acetylation of histone and non-histone proteins, and upregulate heat shock protein 70 and superoxide dismutase,(40–42) both of which are involved in modulation of vitality. Furthermore, VPA treatment has been shown to confer cytoprotection through upregulation of anti-apoptotic proteins and downregulation of pro-apoptotic proteins.(22, 43) Several pro-survival effectors are also modulated by VPA, including Akt, glycogen synthase kinase-3beta, and beta-catenin, to promote cytoprotection and improved survival.(22, 37, 43, 44) As VPA's mechanisms of action appear to broad and diverse, further studies are ongoing to help provide a more comprehensive understanding.

Overall, this study contributes to the growing body of literature supporting VPA as an adjunctive treatment for HS. This study demonstrates that administration of VPA improves survival, decreases resuscitation requirements, and confers favorable laboratory and biochemical profiles following lethal hemorrhage. Most importantly, it is the first study to demonstrate that the protection conferred by a single dose of VPA extends to 72 hours. In a resource-constrained PFC scenario, VPA treatment appears to be a promising option to keep the injured alive when delays in definitive care are anticipated.

There are several limitations to this study. First, although swine are commonly used for translational studies, they are less than perfect surrogates for human subjects. Second, animals underwent extubation at different times. We recognize that this introduces an uncontrolled variable, but it also makes the study more clinically realistic. Furthermore, isoflurane anesthesia can alter the hemodynamic parameters, which would have affected our DCR protocol (aiming a SBP target). However, it would have been unethical to perform these procedures in un-anaesthetized animals. Third, due to limitations of monitoring in awake and mobile animals, blood pressure monitoring occurred only every 2 hours following extubation. Therefore, we did not continuously collect all SBPs which could have potentially qualified for DCR fluid administration. Fourth, we recognize that the DCR protocol used in this study may not reflect resources available in different field care settings. We designed the DCR protocol for this experiment based on the TCCC guidelines, and used only crystalloid and limited colloid resuscitation (without any blood products and dried plasma) to simulate a very resource-limited environment. Lastly, a delay of 72 hours before blood transfusion following injuries may overestimate the length of PFC in most settings; however, we believe that the benefits of VPA can be translated to shorter term models.

In conclusion, this study shows that the addition of VPA to a DCR protocol significantly improves survival and decreases fluid requirements in a 72-hour model of PFC. VPA treatment also improves hemodynamic, laboratory, and biochemical profiles following lethal hemorrhage. As providing care in austere environments and far forward settings is often complicated by delays in evacuation and logistical constraints, VPA represents a promising adjunct to DCR that could maintain survival when delays in definitive care are expected.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Florence C, Haegerich T, Simon T, Zhou C, Luo F. Estimated Lifetime Medical and Work-Loss Costs of Emergency Department-Treated Nonfatal Injuries--United States, 2013. MMWR Morb Mortal Wkly Rep. 2015;64(38):1078–82. [PubMed: 26421663]
2. Florence C, Simon T, Haegerich T, Luo F, Zhou C. Estimated Lifetime Medical and Work-Loss Costs of Fatal Injuries--United States, 2013. MMWR Morb Mortal Wkly Rep. 2015;64(38):1074–7. [PubMed: 26421530]
3. World Health Organization. Injuries and violence: the facts 2014 http://www.who.int/violence_injury_prevention/media/news/2015/Injury_violence_facts_2014/en/ Last accessed 12.21.18.
4. Trunkey DD. Trauma care systems. EmergMed Clini North Am. 1984;2(4):913–22.

5. Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, Uribe P, Mallett O, Zubko T, Oetjen-Gerdes L, Rasmussen TE, et al. Death on the battlefield (2001–2011): implications for the future of combat casualty care. J Trauma Acute Care Surg. 2012;73(6 Suppl 5):S431–7. [PubMed: 23192066]
6. Eastridge BJ, Hardin M, Cantrell J, Oetjen-Gerdes L, Zubko T, Mallak C, Wade CE, Simmons J, Mace J, Mabry R, et al. Died of wounds on the battlefield: causation and implications for improving combat casualty care. J Trauma. 2011;71(1 Suppl):S4–8. [PubMed: 21795876]
7. Butler FK, Holcomb JB, Schreiber MA, Kotwal RS, Jenkins DA, Champion HR, Bowling F, Cap AP, Dubose JJ, Dorlac WC, et al. Fluid Resuscitation for Hemorrhagic Shock in Tactical Combat Casualty Care: TCCC Guidelines Change 14–01−−2 June 2014. J Spec Oper Med. 2014;14(3):13–38
8. Baraniuk S, Tilley BC, del Junco DJ, Fox EE, van Belle G, Wade CE, Podbielski JM, Beeler AM, Hess JR, Bulger EM, et al. Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) Trial: design, rationale and implementation. Injury. 2014;45(9):1287–95. [PubMed: 24996573]
9. Powell EK, Hinckley WR, Gottula A, Hart KW, Lindsell CJ, McMullan JT. Shorter times to packed red blood cell transfusion are associated with decreased risk of death in traumatically injured patients. J Trauma Acute Care Surg. 2016;81(3):458–62. [PubMed: 27050884]
10. Falk JL, Rackow EC, Weil MH. Colloid and crystalloid fluid resuscitation. Acute Care. 1983;10(2):59–94. [PubMed: 6085668]
11. Ross SW, Christmas AB, Fischer PE, Holway H, Seymour R, Huntington CR, Heniford BT, Sing RF. Defining Dogma: Quantifying Crystalloid Hemodilution in a Prospective Randomized Control Trial with Blood Donation as a Model for Hemorrhage. J Am Coll Surg. 2018;227(3):321–31. [PubMed: 29879520]
12. Rizoli SB. Crystalloids and colloids in trauma resuscitation: a brief overview of the current debate. J Trauma. 2003;54(5 Suppl):S82–8. [PubMed: 12768108]
13. Ho AM, Karmakar MK, Contardi LH, Ng SS, Hewson JR. Excessive use of normal saline in managing traumatized patients in shock: a preventable contributor to acidosis. J Trauma. 2001;51(1):173–7. [PubMed: 11468491]
14. Cotton BA, Guy JS, Morris JA Jr, Abumrad NN. The cellular, metabolic, and systemic consequences of aggressive fluid resuscitation strategies. Shock. 2006;26(2):115–21. [PubMed: 16878017]
15. Haupt MT, Teerapong P, Green D, Schaeffer RC Jr, RW Carlson. Increased pulmonary edema with crystalloid compared to colloid resuscitation of shock associated with increased vascular permeability. Circ Shock. 1984;12(3):213–24. [PubMed: 6713602]
16. DeSoucy E, Shackelford S, DuBose JJ, Zweben S, Rush SC, Kotwal RS, Montgomery HR, Keenan S. Review of 54 Cases of Prolonged Field Care. JSpec Oper Med.17(1):121–9. [PubMed: 28285490]
17. Keenan S, Riesberg JC. Prolonged Field Care: Beyond the “Golden Hour”. Wilderness Environ Med. 2017;28(2s):S135–s9. [PubMed: 28601206]
18. Joint Program Committee 6/Combat Casualty Care Research Program. Prolonged Field Care Research Award. https://cdmrp.army.mil/funding/pa/16dmrdpcccrppfcra_pa.pdf Last accessed 12.30.18.
19. Halaweish I, Nikolian V, Georgoff P, Li Y, Alam HB. Creating a “Prosurvival Phenotype” Through Histone Deacetylase Inhibition: Past, Present, and Future. Shock. 2015;44 Suppl 1:6–16. [PubMed: 25565645]
20. Shults C, Sailhamer EA, Li Y, Liu B, Tabbara M, Butt MU, Shuja F, Demoya M, Velmahos G, Alam HB. Surviving blood loss without fluid resuscitation. J Trauma. 2008;64(3):629–38; discussion 38–40. [PubMed: 18332802]
21. Alam HB, Hamwi KB, Duggan M, Fikry K, Lu J, Fukudome EY, Chong W, Bramos A, Kim K, Velmahos G. Hemostatic and pharmacologic resuscitation: results of a long-term survival study in a swine polytrauma model. J Trauma. 2011;70(3):636–45. [PubMed: 21610354]
22. Alam HB, Shuja F, Butt MU, Duggan M, Li Y, Zacharias N, Fukudome EY, Liu B, Demoya M, Velmahos GC. Surviving blood loss without blood transfusion in a swine poly-trauma model. Surgery. 2009;146(2):325–33. [PubMed: 19628092]

23. Liu Z, Li Y, Chong W, Deperalta DK, Duan X, Liu B, Halaweish I, Zhou P, Alam HB. Creating a prosurvival phenotype through a histone deacetylase inhibitor in a lethal two-hit model. Shock. 2014;41(2):104–8. [PubMed: 24430491]
24. Kim K, Li Y, Jin G, Chong W, Liu B, Lu J, Lee K, Demoya M, Velmahos GC, Alam HB. Effect of valproic acid on acute lung injury in a rodent model of intestinal ischemia reperfusion. Resuscitation. 2012;83(2):243–8. [PubMed: 21824465]
25. Causey MW, Miller S, Hoffer Z, Hempel J, Stallings JD, Jin G, Alam H, Martin M. Beneficial effects of histone deacetylase inhibition with severe hemorrhage and ischemia-reperfusion injury. JSurgRes. 2013;184(1):533–40.
26. Halaweish I, Bambakidis T, Chang Z, Wei H, Liu B, Li Y, Bonthrone T, Srinivasan A, Bonham T, Chtraklin K, et al. Addition of low-dose valproic acid to saline resuscitation provides neuroprotection and improves long-term outcomes in a large animal model of combined traumatic brain injury and hemorrhagic shock. J Trauma Acute Care Surg. 2015;79(6):911–9; discussion 9. [PubMed: 26680134]
27. Nikolian VC, Georgoff PE, Pai MP, Dehaney IS, Chtraklin K, Eidy H, Ghandour MH, Han Y, Srinivasan A, Li Y, et al. Valproic acid decreases brain lesion size and improves neurologic recovery in swine subjected to traumatic brain injury, hemorrhagic shock, and polytrauma. J Trauma Acute Care Surg. 2017; 83(6):1066–1073. [PubMed: 28697014]
28. Jepsen CH, deMoya MA, Perner A, Sillesen M, Ostrowski SR, Alam HB, Johannsson PI. Effect of valproic acid and injury on lesion size and endothelial glycocalyx shedding in a rodent model of isolated traumatic brain injury. J Trauma Acute Surg. 2014;77(2):292–7.
29. Holcomb JB. Damage control resuscitation. The Journal of trauma. 2007;62(6 Suppl):S36–7. [PubMed: 17556961]
30. Duchesne JC, McSwain NE Jr., Cotton BA, Hunt JP, Dellavolpe J, Lafaro K, Marr AB, Gonzalez EA, Phelan HA, Bilski T, et al. Damage control resuscitation: the new face of damage control. J Trauma. 2010;69(4):976–90. [PubMed: 20938283]
31. Butler FK Jr, Fluid Resuscitation in Tactical Combat Casualty Care: Yesterday and Today. Wilderness Environ Med. 2017;28(2s):S74–s81. [PubMed: 28601214]
32. Montgomery HR, Butler FK, Kerr W, Conklin CC, Morissette DM, Remley MA, Shaw TA, Rich TA. TCCC Guidelines Comprehensive Review and Update: TCCC Guidelines Change 16–03. JSpec OperMed.2017(2):21–38.
33. Baker BL, Powell D, Riesberg J, Keenan S. Prolonged Field Care Working Group Fluid Therapy Recommendations. J Spec Oper Med. 2016;16(1):112–7. [PubMed: 27045508]
34. Keenan S Prolonged Field Care Working Group Update. J Spec Oper Med. 2016;16(2):105–6. [PubMed: 27450613]
35. Hu S, Hou JY, Li L, Yang MX, Sheng ZY. [The effects of administration of valproic acid on organ function and outcome in a canine lethal hemorrhagic shock model]. Zhongguo Wei ZhongBing Ji Jiu Yi Xue. 2011;23(8):471–4.
36. Hwabejire JO, Lu J, Liu B, Li Y, Halaweish I, Alam HB. Valproic acid for the treatment of hemorrhagic shock: a dose-optimization study. JSurgRes. 2014;186(1):363–70.
37. Zacharias N, Sailhamer EA, Li Y, Liu B, Butt MU, Shuja F, Velmahos GC, De Moya M, Alam HB. Histone deacetylase inhibitors prevent apoptosis following lethal hemorrhagic shock in rodent kidney cells. Resuscitation. 2011;82(1):105–9. [PubMed: 21036453]
38. Rocha Filho JA, Nani RS, D'Albuquerque LA, Holms CA, Rocha JP, Sa Malbouisson LM, Machado MC, Carmona MJ, Auler Juior JO. Hyperkalemia accompanies hemorrhagic shock and correlates with mortality. Clinics (SaoPaulol). 2009;64(6):591–7.
39. Rocha Filho JA, Nani RS, D'Albuquerque LA, Malbouisson LM, Carmona MJ, Rocha ESM, Auler JO. Potassium in hemorrhagic shock: a potential marker of tissue hypoxia. The J Trauma. 2010;68(6):1335–41. [PubMed: 20539177]
40. Gonzales E, Chen H, Munuve R, Mehrani T, Britten-Webb J, Nadel A, Alam HB, Wherry D, Burris D, Koustava E. Valproic acid prevents hemorrhage-associated lethality and affects the acetylation pattern of cardiac histones. Shock. 2006;25(4):395–401. [PubMed: 16670643]

41. Lin T, Alam HB, Chen H, Britten-Webb J, Rhee P, Kirkpatrick J, Koustova E. Cardiac histones are substrates of histone deacetylase activity in hemorrhagic shock and resuscitation. Surgery. 2006;139(3):365–76. [PubMed: 16546502]

42. Lin T, Chen H, Koustova E, Sailhamer EA, Li Y, Shults C, Liu b, Rhee P, Kirkpatrick J, Alam HB. Histone deacetylase as therapeutic target in a rodent model of hemorrhagic shock: effect of different resuscitation strategies on lung and liver. Surgery. 2007;141(6):784–94. [PubMed: 17560255]

43. Chang P, Weykamp M, Dennahy IS, Williams AM, Bhatti UF, Liu B, Nikolian VC, Li Y, Alam HB. Histone deacetylase inhibitors: Isoform selectivity improves survival in a hemorrhagic shock model. J Trauma Acute Care Surg. 2018;84(5):795–801. [PubMed: 29401190]

44. Li Y, Liu B, Sailhamer EA, Yuan Z, Shults C, Velmahos GC, deMoya M, Shuja F, Butt MU, Alam HB. Cell protective mechanism of valproic acid in lethal hemorrhagic shock. Surgery. 2008;144(2):217–24. [PubMed: 18656628]

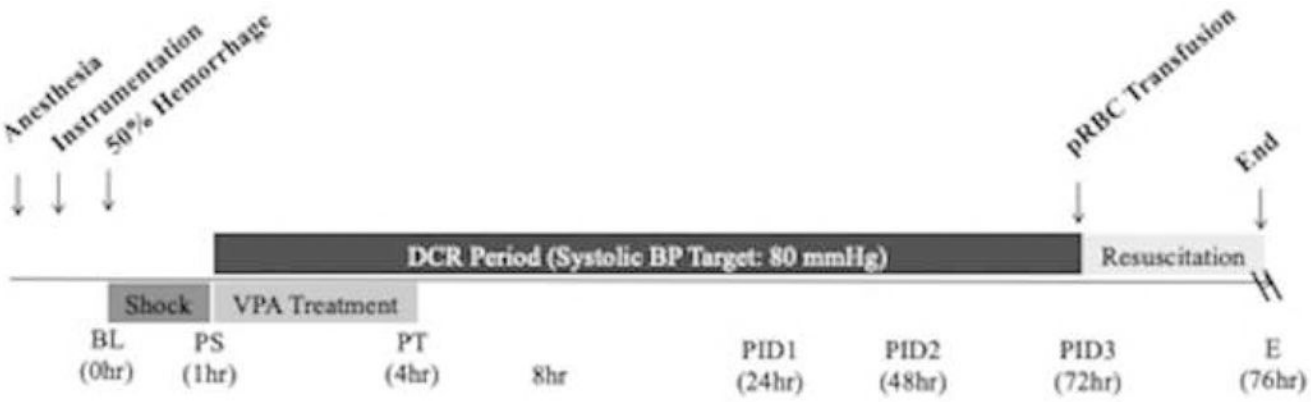

Figure 1. Visual representation of experimental model.

Injury model consisting of 50% estimated total blood volume hemorrhage. Animals are left in shock for 1 hour following completion of hemorrhage. Animals are then randomized to hemorrhagic shock (HS) alone, HS-damage control resuscitation (DCR), and HS-DCR-valproic acid (VPA). Animals then undergo 72 hours of prolonged DCR. At 72 hours, surviving animals receive transfusion of autologous packed red blood cells, simulating transfer to a higher echelon of care. Additional resuscitation is administered as needed until return to baseline laboratory parameters. *DCR.,* damage control resuscitation; *VPA,* valproic acid; *pRBC,* packed red blood cells; *BL,* baseline; *PS,* post-shock; *PT*, post-treatment; *PID*, post-injury day; *E*, end.

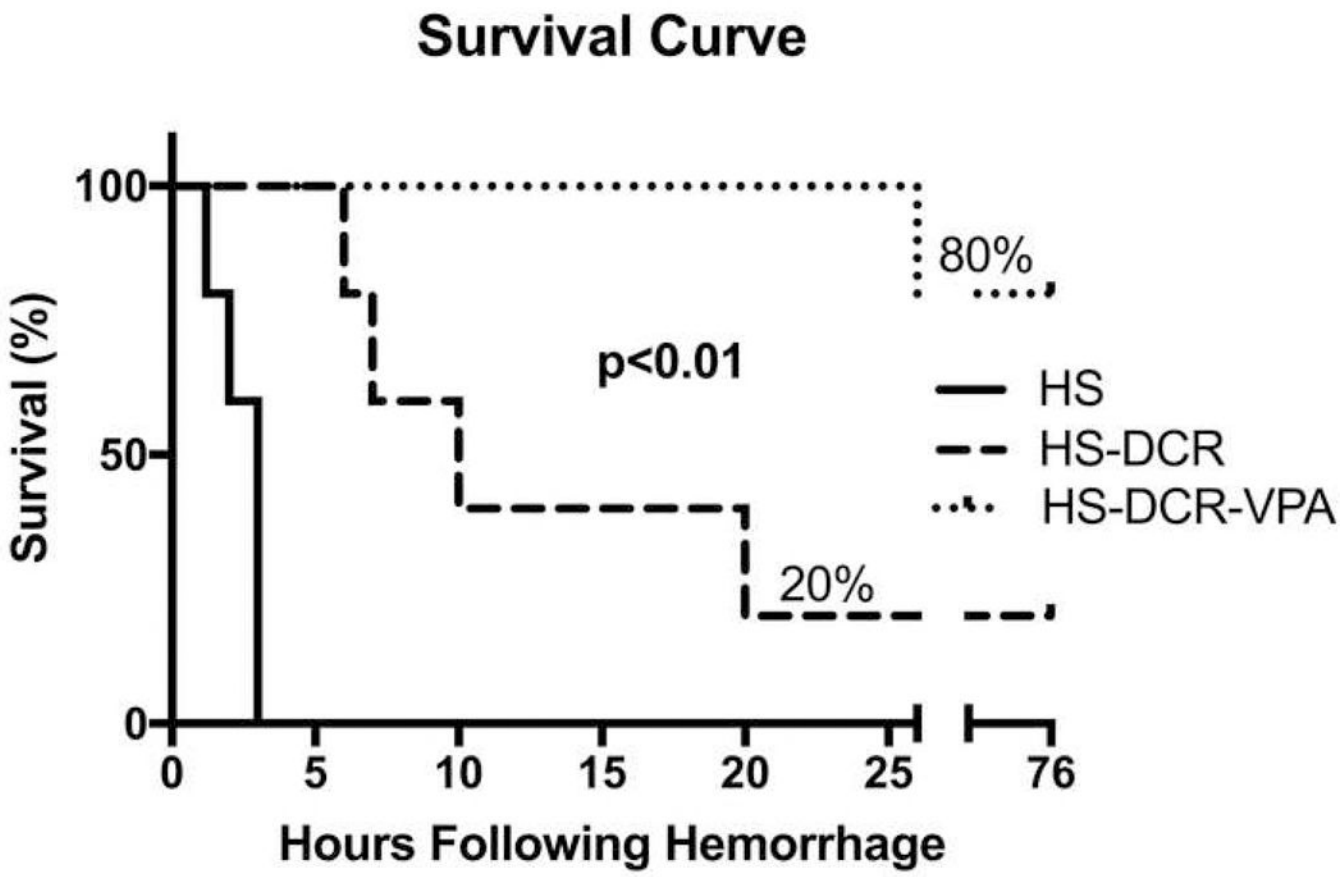

Figure 2. Kaplan Meier curve of survival following hemorrhage.

Kaplan Meir survival curve showing survival rates in the HS, HS-DCR, and HS-DCR-VPA groups according to time following hemorrhage. Survival rate expressed as number of animals surviving until the end of the 76-hour experiment. All animals in the HS group died before the end of the experiment. Treatment with DCR and DCR-VPA increased survival to 20% and 80%, respectively ($p < 0.01$). *HS,* hemorrhagic shock; *DCR,* damage control resuscitation; *VPA,* valproic acid.

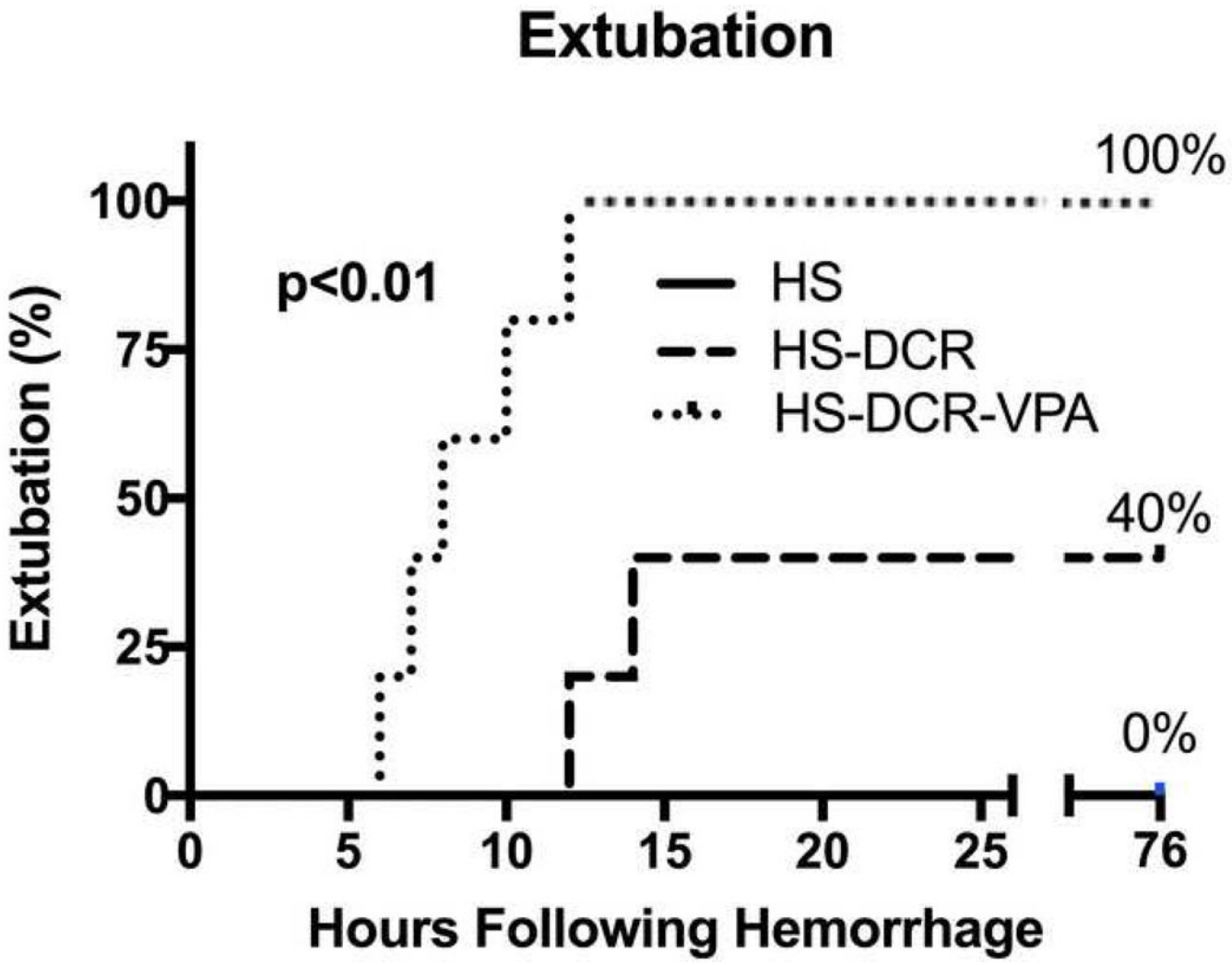

Figure 3. Kaplain Meier curve showing timing and rates of extubation following hemorrhage.

Kaplan Meir curve showing extubation rates in the HS, HS-DCR, and HS-DCR-VPA groups according to time following hemorrhage. Extubation rates expressed as animals meeting physiologic criteria for extubation. None of the animals in the HS group met criteria for extubation. Treatment with DCR and DCR-VPA increased extubation rates to 40% and 100%, respectively ($p < 0.01$). Animals in the HS-DCR-VPA group were extubated earlier than animals in the HS-DCR group. *HS,* hemorrhagic shock; *DCR,* damage control resuscitation; *VPA,* valproic acid.

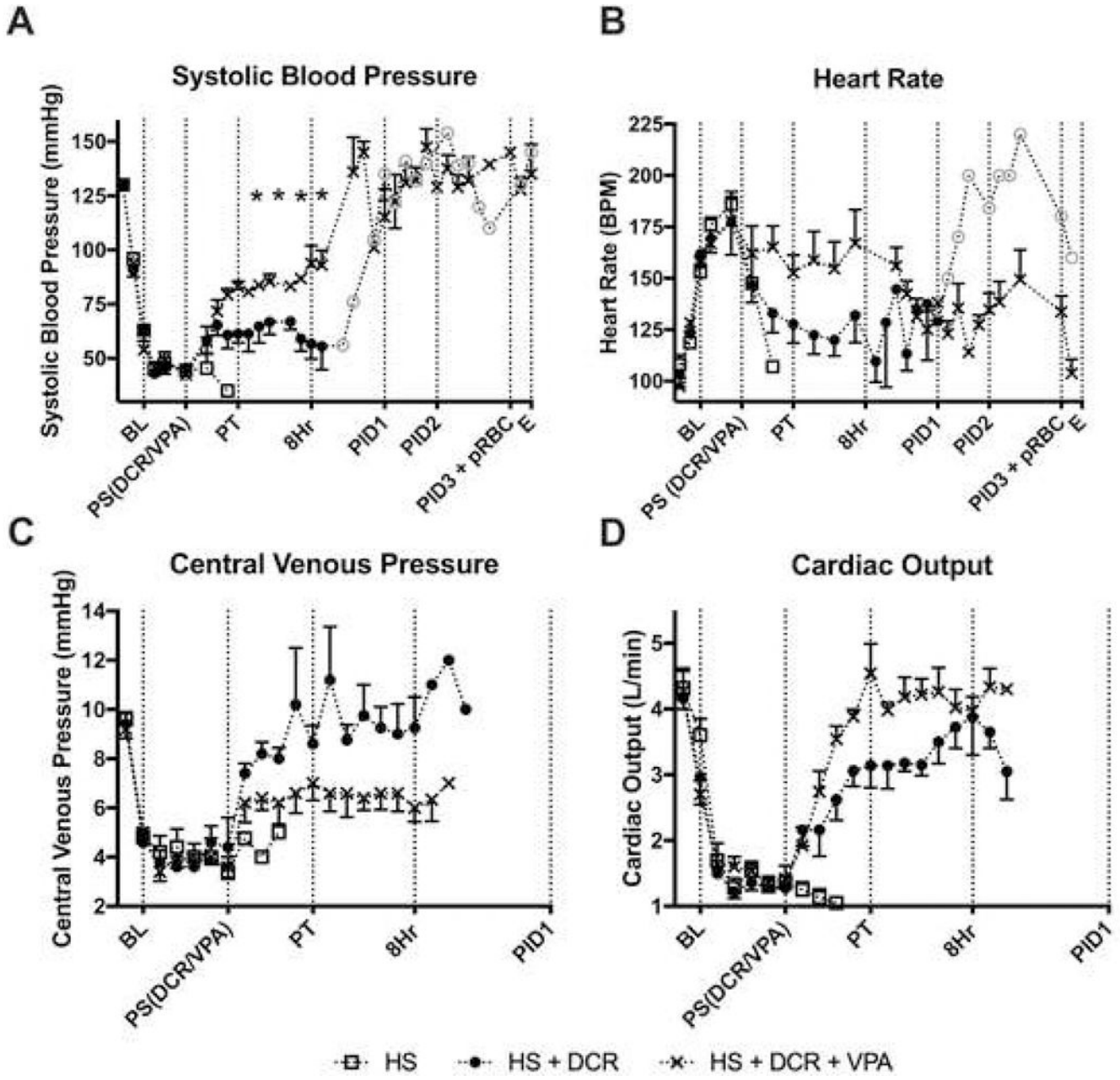

Figure 4. Hemodynamic data throughout the experiment.

Intraoperative measurements of (A) heart rate, (B) systolic blood pressure, (C) central venous pressure, and (D) cardiac output. Data are presented as group mean ± standard error. Asterisks indicate discrete time points which were significantly different ($p < 0.05$). Grey circles represent the time points for which n=1 in the HS-DCR group. HS-DCR-VPA animals had significantly increased SBP at several discrete time points. However, no significant differences were noted in other hemodynamic responses. HS – hemorrhagic shock; DCR – damage control resuscitation; VPA – valproic acid; BL – baseline; PS – post-shock; PID – postinjury day; pRBC – packed red blood cells; E – end; BPM-beats per minute.

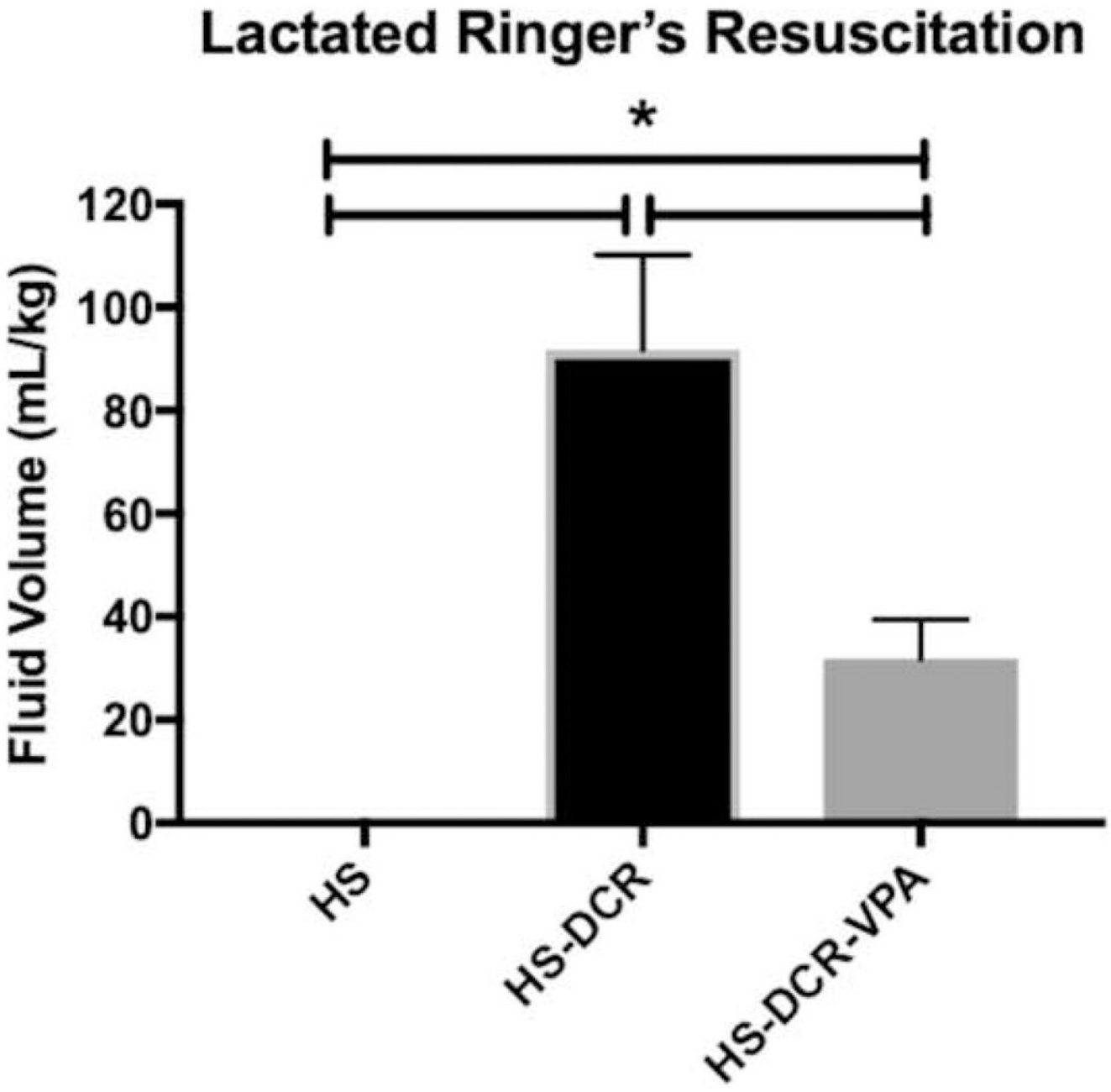

Figure 5. Resuscitation requirements of each group during the prolonged field care stage of the experiment.

Graph showing total volume infused of Lactated Ringer's solution as part of damage control resuscitation. Boluses were administered to meet systolic blood pressure requirements of 80mm Hg. Note that animals in the HS group did not receive LR as they were not assigned to receive DCR. Animals in the HS-DCR-VPA group had significantly decreased LR requirements compared to the HS-DCR group (LR fluid resuscitation, ml/kg – HS-DCR: 91.8 ± 18.3 ml/kg; HS-DCR-VPA: 31.8 ± 7.6 ml/kg; $p<0.001$). *HS,* hemorrhagic shock; *DCR,* damage control resuscitation; *VPA,* valproic acid.

Table 1.

Extubation Parameters

Parameters	Criteria
Partial Pressure of Oxygen	> 60 mmHg
Partial Pressure of Carbon Dioxide	35–45 mmHg
Systolic Blood Pressure	> 80 mm Hg for 1 hour without additional fluid boluses
Acid-Base Status	pH > 7.25
Lactate Level	< 8 mM
Neurologic Status	Intact cough/gag reflex Spontaneous movement of all extremities

Table 2.

Select Laboratory Parameters

		Time Points			
		BL	**PS**	**PT**	**8Hr**
pH	HS	7.44 (0.03)	7.37 (0.05)		
	HS-DCR	7.41 (0.02)	7.38 (0.04)	7.33 (0.06)	7.37 (0.06)
	HS-DCR-VPA	7.45 (0.02)	7.36 (0.03)	7.32 (0.05)	7.44 (0.04)
pCO_2 (mmHg)	HS	43.1 (4.1)	36 (3.7)		
	HS-DCR	42.5 (2.9)	39.6 (4.9)	41.4 (3.9)	38.4 (2.8)
	HS-DCR-VPA	39.3 (2.6)	41.0 (4.2)	43.9 (2.7)	42.8 (3.0)
sO_2 (%)	HS	118.7 (15.7)	109.7 (44.5)		
	HS-DCR	130 (14.5)	103.9 (39.3)	132.5 (33.5)	113.4 (21.4)
	HS-DCR-VPA	135.2 (17.9)	102.1 (39.1)	129.6 (30.0)	92.4 (21.0)
Na (mM)	HS	137 (2.1)	135 (2.5)		
	HS-DCR	138 (2.9)	136 (2.4)	137 (2.2)	136 (3.3)
	HS-DCR-VPA	138 (3.9)	137 (1.8)	139 (1.5)	139 (2.2)
K (mM)	HS	4.1 (0.4)	5.4 (1.4)		
	HS-DCR	4.3 (0.5)	4.8 (0.6)	5.1 (1.0)	**6.6 (0.5)** *
	HS-DCR-VPA	4.7 (0.6)	5.2 (0.5)	5.0 (1.0)	**5.4 (0.4)** *
Gluc (mg/dl)	HS	88.7 (13.8)	89 (31)		
	HS-DCR	90 (18)	96 (25)	78 (16)	124 (16)
	HS-DCR-VPA	78 (15)	109 (17)	96 (24)	155 (33)
Hb (g/dL)	HS	9.1 (0.8)	9.7 (1.5)		
	HS-DCR	8.6 (0.9)	8.9 (1.4)	5.2 (1.0)	**5.9 (1.1)** *
	HS-DCR-VPA	8.6 (0.7)	8.1 (1.5)	5.9 (1.3)	**8.2 (0.8)** *
Lac (mM)	HS	1.6 (0.5)	7.9 (2.8)		
	HS-DCR	1.5 (0.5)	7.7 (1.4)	4.9 (2.4)	5.2 (2.8)
	HS-DCR-VPA	1.5 (0.4)	6.9 (1.5)	6.1 (2.1)	7.5 (2.3)

Data presented as group mean with standard deviation (SD) shown in parentheses.

*designates $p < 0.05$. Statistically significant results are shown in bold. HS = hemorrhagic shock; DCR = damage control resuscitation; VPA = valproic acid; BL = baseline; PS = post-shock (onset of DCR and VPA); PT = post-treatment (VPA); 8Hr = eight hours following injuries; pCO2 = partial pressure of CO_2; Hb = hemoglobin; sO_2 = O_2 saturation; Na = sodium; K = potassium; Gluc = glucose; Hb = hemoglobin; Lac = lactate.

ANOVA was used for all comparisons among three groups. Students i-test was used for comparisons between two groups (PT and 8Hr time-point).