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TITLE: Aortic Hemostasis and Resuscitation: Advanced REBOA for NCTH and Reversal of HiTCA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The majority of combat and civilian casualties are due to severe uncontrolled, non-compressible hemorrhage resulting in cardiovascular collapse. Current resuscitation techniques, including cardiopulmonary resuscitation (CPR), thoracotomy, and aortic cross-clamping to reverse hemorrhage-induced traumatic cardiac arrest (HiTCA), are very invasive and ineffective for austere combat casualties. Aortic Hemostasis and Resuscitation (AHR) is advanced form of resuscitative endovascular balloon occlusion of the aorta (REBOA) that has been recently shown to promote return of spontaneous circulation (ROSC). This study examines the survival benefit of AHR in otherwise fatal non-compressible torso hemorrhage (NCTH) with HiTCA. It aims to compare the efficacy of the selective aortic arch perfusion (SAAP) catheter when used with fresh whole blood or an oxygen therapeutic (HBOC). In addition, it aims to demonstrate the feasibility of the conversion from SAAP therapy to limited extra-corporeal life support (ECLS), and also to determine the impact of ECLS on critical physiology. To do this, we utilized a model of swine NCTH and HiTCA in a series of experiments in which each animal underwent a liver laceration and allowed to free bleed for five minutes through the SAAP catheter to achieve HiTCA. Then, resuscitation fluid is selectively perfused through the balloon catheter to the heart and brain, while also limiting non-compressible bleeding below the balloon. As a result, ROSC was achieved in 100% of the FWB animals and 86% of the HBOC-201 animals (p=0.12). Overall survival (t = 320 min) was 92% in the FWB group and 67% in the HBOC-201 group (p=0.12). This study shows that the SAAP catheter is an effective method of hemorrhage control by promoting ROSC and sustaining life in pre-hospital transport. AHR promotes hemodynamic stability and has the potential to fill a critical unmet gap in military and civilian trauma care.

15. SUBJECT TERMS
Non-compressible torso hemorrhage (NCTH), hemorrhage-induced traumatic cardiac arrest (HiTCA), resuscitative endovascular balloon occlusion of the aorta (REBOA), aortic hemostasis and resuscitation (AHR), hemoglobin-based oxygen carrier (HBOC), selective aortic arch perfusion (SAAP), extracorporeal life support (ECLS), extracorporeal membrane oxygenation (ECMO)

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Unclassified	Unclassified	Unclassified			

TABLE OF CONTENTS

Page No.

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope

Uncontrolled, non-compressible hemorrhage resulting in cardiovascular collapse accounts for the majority of civilian and military casualties. Current techniques used against hemorrhage-induced cardiac arrest in severe combat casualty care have been shown to be ineffective, thus survival rates are low. In contrast, aortic hemostasis and resuscitation (AHR) has been shown to be effective in hemorrhage control. AHR with oxygenated whole blood and packed red blood cells has been shown to stimulate the return of spontaneous circulation (ROSC). SAAP is a technique in which a balloon catheter is introduced into the aorta and inflated to stop bleeding. This study aims to evaluate the effectiveness of selective aortic arch perfusion (SAAP) when used in combination with an external pump system that infuses either fresh whole blood or hemoglobin-based oxygen carrier (HBOC) to provide the body with oxygen after circulation is restored. The main objective is to demonstrate the survival benefit of AHR using a large animal model by effectively achieving ROSC, providing hemodynamic support, and increase pre-hospital transport and long-term survival.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Non-compressible torso hemorrhage (NCTH), hemorrhage-induced traumatic cardiac arrest (HiTCA), resuscitative endovascular balloon occlusion of the aorta (REBOA), aortic hemostasis and resuscitation (AHR), hemoglobin-based oxygen carrier (HBOC), selective aortic arch perfusion (SAAP), extracorporeal life support (ECLS)

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1/Phase I (Y1)
Subtask 1: Submit documents for IACUC approval
Milestone # 1 IACUC approval obtained (3/16/2016)
Subtask 2: Submit documents for ACURO approval
Milestone # 2 ACURO approval obtained (10/24/2016)
Subtask 3: Staff Hiring (Q2)
Subtask 4: Surgical capability Start-up including 6 pilot/model refinement animals
Subtask 5: Phase I study execution (two experimental groups): animal experiments – fresh whole SAAP vs HBOC SAAP with critical care observation and limited ECLS as per protocol 24 with schedule for up to 4 technical failures experimental swine total (2 pig per 1-2 weeks); 60 donor swine (Completed 5/5/2017)
Subtask 6: QA of data entry, statistical analysis, and final study report (Completed 7/13/2018)

Major Task 2/Phase II (Y2)
Subtask 1: Development of didactic training component and recruit first class of five participants. (In Progress)
Subtask 2: Complete first AHR course. Assess and refine coursework. 16 swine for training; 30 donor swine. (In Progress)
Subtask 3: Recruit second class of five participants and complete second AHR course.
Subtask 4: QA of data entry, statistical analysis, and final study report.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in this section should shift from reporting activities to reporting accomplishments.

1) Phase I experiments and data analysis have been completed in preparation of Y2/Phase II of training development. Y2/Phase II didactic training component is in preparation, along with recruiting for the first AHR course.

2) The objectives were to compare the efficacy of SAAP therapy between oxygenated HBOC and oxygenated FWB, and also to determine if SAAP can be converted to SAAP-ECMO therapy without negatively impacting critical physiology achieved by the aortic balloon occlusion.
In this study, the SAAP catheter is introduced into the aorta and inflated to stop bleeding while allowing the administration of oxygenated resuscitation from an external pump system directly into the heart during cardiac arrest to achieve ROSC.

3) The results show that HBOC-201 and fresh whole blood are both effective at promoting ROSC in HiTCA and that the conversion from SAAP to ECMO via the SAAP catheter is feasible.
All FWB animals achieved ROSC, while 12 of the 14 HBOC animals achieved ROSC. Five animals (4 HBOC, 1 FWB) died before the end of experiment. All animals except one that achieved ROSC survived pre-hospital and converted to ECLS. A full manuscript of results are included in the appendix of this document.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Level II/III emergency care providers will be trained in the AHR technique in Y2 to demonstrate its military applicability and survival benefit prior to clinical use.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

An abstract from the Phase I data has been accepted as an oral presentation at the annual meeting for the American Association for the Surgery of Trauma. It will be presented on 9/27/2018. A secondary manuscript is in progress describing the physiologic sequalae of the injury complex.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Complete the didactic training component, recruit participants, and train the emergency providers in the technique to demonstrate its feasibility. The training will be a combination of didactic lectures, vascular access training, and labs, using a non-recovered swine model of NCTH and HiTCA.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The aim is for the results to demonstrate that AHR improves ROSC and survival of patients in pre-hospital transport, and ultimately in long-term survival. This likely could change the standard of hemorrhage control and resuscitation strategies.

In addition, HBOC has a longer shelf-life than fresh whole blood and may be a future alternative for human erythrocytes. It can be kept at room temperature for up to three years, does not have to be matched with blood type, and can be used on patients with immune systems that attack red blood cells. HBOC could potentially eliminate the need for whole blood in the use of SAAP technology, extending its pre-hospital viability in combat theaters of operation.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Primary impact is the potential adoption of a new resuscitation technique in military and civilian care. Results, use and training algorithms, and new practices will be transferred to both civilian and military centers through training programs and involvement of both civilian and military medical centers in upcoming clinical trials.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

SAAP and AHR have been shown to increase the likelihood of achieving ROSC, thus survival rates of patients will likely increase, which will decrease the number of fatalities after non-compressible torso hemorrhage (NCTH) and hemorrhage induced traumatic cardiac arrest (HiTCA).

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

An IRB waiver was completed on March 26, 2018.

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

American Association for the Surgery of Trauma Annual Meeting abstract submission March 1, 2018. Manuscript submitted to Journal of Trauma.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

  Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Presented at Baylor University Medical Center's Grand Rounds in Dallas May 11, 2017

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- *Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.*

The primary product is the AHR/SAAP catheter technique which uses an oxygenated fluid to achieve ROSC in order to sustain post-ROSC survival until vascular control of hemorrhage occurs to promote overall long-term survival. SAAP technology is owned by the University of North Carolina and by Resuscitech INC. Resuscitech INC is in the process of manufacturing and pre-FDA submission for SAAP catheters. Use of the SAAP technique in HiTCA will be shared via the training program developed in the second year of this project in addition to worldwide speaking engagements and clinical trials.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

The primary reportable outcome of the first phase of this project is an enhanced understanding of the following: 1) use of non-blood product substrates with SAAP perfusion and 2) technical feasibility of transition from SAAP to SAAP-ECLS for cardiac support during critical care.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project? *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project*

during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

Name: James D Ross, PhD
Project Role: PI
Research Identifier: NA
Nearest Person Month Worked: 3
Contribution to Project: ACURO documentation preparation and submission. Pilot experiment execution. Pilot data evaluation. Report preparation. Control administration of blood products. Monitor animals during experiments. Full study execution including surgical procedures, ECMO circuit/pump management and critical care decision making.

Name: James Manning, MD
Project Role:
Research Identifier: NA
Nearest Person Month Worked: 4
Contribution to Project: Developer of SAAP catheter. Monitor animals during experiment. Critical care decision making. Analysis of data and outcomes.

Name: Todd Graham
Project Role: Research Technician
Research Identifier: NA
Nearest Person Month Worked: 9
Contribution to Project: Laboratory equipment set-up and maintenance. Coordination of equipment training protocols. Development of BIOPAC data acquisition templates. Development of consumable supply ordering schedules for phase I. Execution of laboratory experiments. Coordination of data review. DEA licensure coordination and schedule drug ordering.

Name: Lauren Wilson
Project Role: Research Technician
Research Identifier: NA
Nearest Person Month Worked: 9
Contribution to Project: Production of run-sheets and laboratory/OR scheduling and turnover for final experiments. Loading blood products for administration. Laboratory equipment maintenance. Pump technology testing and calibration.

Name: Brianne Madtson
Project Role: Research Technician
Research Identifier: NA
Nearest Person Month Worked: 3
Contribution to Project: Animal schedule coordination. Consumable supply ordering. Laboratory set-up and equipment maintenance.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

The project included a subaward collaboration with the Co-PI, Dr. James Manning, and his laboratory at University of North Carolina as detailed in the contract subaward. All experiments were performed on-site at Oregon Health and Science University.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A

Selective Aortic Arch Perfusion with fresh whole blood or HBOC-201 reverses hemorrhage-induced traumatic cardiac arrest in a lethal model of non-compressible torso hemorrhage

[1]Heather E Hoops MD, [2]James E Manning MD, [3]Todd L Graham BS, [3]Belinda H McCully PhD, [2]Shane L. McCurdy BS, [3]James D Ross PhD

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Presented at: American Association for the Surgery of Trauma 2018 Annual Meeting in San Diego, CA.

Short Title: SAAP reliably reverses traumatic cardiac arrest

Body Word Count: 3999

SOURCES OF FUNDING

This body of work was funded by the Defense Medical Research and Development Program. Award number: W81XWH-16-2-0012

DISCLOSURES

JEM declares intellectual property interests in SAAP technology. He is also the co-founder of Resusitech, Inc., a medical device company developing SAAP technologies. JEM has also delivered approximately 30 invited lectures on SAAP for resuscitation in medical and traumatic cardiac arrest. For several lectures, travel costs were reimbursed by the inviting organization or the University of North Carolina.

BACKGROUND:

Hemorrhage-induced traumatic cardiac arrest (HiTCA) has a dismal survival rate. Previous studies demonstrated selective aortic arch perfusion (SAAP) with fresh whole blood (FWB) improved the rate of return of spontaneous circulation (ROSC) after HiTCA, compared to REBOA and CPR. Hemoglobin-based oxygen carriers, such as HBOC-201, may alleviate the logistical constraints of using FWB in a prehospital setting. It is unknown whether SAAP with HBOC-201 is equivalent in efficacy to FWB, whether conversion from SAAP to Extracorporeal Life Support (ECLS) is feasible, and whether physiologic derangement post-SAAP therapy is reversible.

METHODS:

Twenty-six swine (79±4kg) were anesthetized and underwent HiTCA which was induced via liver injury and controlled hemorrhage. Following arrest, swine were randomly allocated to resuscitation using SAAP with FWB (n=12) or HBOC-201 (n=14). After SAAP was initiated, animals were monitored for a 20-minute pre-hospital period prior to a 40-minute damage control surgery and resuscitation phase, followed by 260 minutes of critical care. Primary outcomes included rate of ROSC, survival, conversion to ECLS, and correction of physiology.

RESULTS:

Baseline physiologic measurements were similar between groups. ROSC was achieved in 100% of the FWB animals and 86% of the HBOC-201 animals (p=0.483). Survival (t=320-min) was 92% (11/12) in the FWB group and 67% (8/12) in the HBOC-201 group (p=0.120). Conversion to ECLS was successful in 100% of both groups. Lactate peaked at 80 minutes in both groups, and significantly improved by end of experiment in the HBOC-201 group (p=0.001) but not in the FWB group (p=0.104). There was no significant difference in peak or end lactate between groups.

CONCLUSIONS:

SAAP is effective in eliciting ROSC after HiTCA in a swine model, using either FWB or HBOC-201. Transition from SAAP to ECLS after definitive hemorrhage control is feasible, resulting in high overall survival and improvement in lactic acidosis over the study period.

LEVEL OF EVIDENCE: Basic science, therefore this study does not require a defined level of evidence

STUDY TYPE: Therapeutic

KEYWORDS: Resuscitation; traumatic cardiac arrest; *Sus scrofa*; selective aortic arch perfusion, SAAP

BACKGROUND

Hemorrhage is the leading cause of preventable traumatic death in both military and civilian settings, with most deaths occurring prior to medical treatment facility arrival.[1-4] Hemorrhage-induced traumatic cardiac arrest (HiTCA) represents the most severe state of hemorrhagic shock, with a dismal survival rate of 0-20% despite current treatment methods (closed-chest compressions, resuscitative thoracotomy, and concurrent blood product resuscitation).[5-7] Using Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA) to achieve aortic hemostasis has not improved survival rates in HiTCA[8] and may increase mortality.[9] More efficacious treatment options for HiTCA are needed.

Selective aortic arch perfusion (SAAP) is a novel endovascular aortic occlusion technique that provides the same hemostatic effects as REBOA, but can additionally provide intra-aortic perfusion of the brain and coronary arteries for treatment of HiTCA.[10] While REBOA is effective at treating severe hemorrhagic shock in both a swine model and in clinical settings,[8, 11, 12] its efficacy in HiTCA is limited.[8, 13] Previous studies have demonstrated that SAAP using fluid with oxygen carrying capacity is superior to SAAP with Lactated Ringer's,[13, 14] REBOA[13] and closed-chest compressions (unpublished data) with whole blood resuscitation in reversing HiTCA in a swine model.

Blind placement of intra-arterial catheters in patients with cardiac arrest in a pre-hospital setting has been shown to be feasible.[8, 15-17] However, the logistics of using SAAP with fresh whole blood (FWB) presents a challenge in the pre-hospital environment due to storage considerations and citrate-related calcium sequestration, requiring co-administration of exogenous calcium.[14]

While controversial in use associated with elective surgery,[18-20] the benefits of HBOC-201 (HbO2 Therapeutics, Souderton, PA) and other hemoglobin-based oxygen carriers may outweigh risks in settings where blood products are unavailable or unacceptable due to austere medical environments or religious preferences.[21, 22]

SAAP using HBOC-201 has been shown to effectively achieve return of spontaneous circulation (ROSC) after HiTCA in a swine model.[23] HBOC-201 may provide additional benefit in the treatment of HiTCA in austere environments or emergent settings as it does not require calcium co-administration. However, there have been no previous randomized trials comparing the efficacy of SAAP with fresh whole blood to HBOC-201 in the treatment of HiTCA.

After ROSC, ongoing cardiovascular support may be needed due to severe cardiac dysfunction and systemic physiologic derangements from ischemia-reperfusion injury. Veno-arterial extracorporeal Life Support (ECLS) has been successfully used in trauma patients with pre-hospital or in-hospital HiTCA,[24, 25] with a published survival rate of 42-63% in a recent systematic review.[26]

We hypothesized that there will be similar rates of ROSC and overall survival after HiTCA treated with SAAP therapy using either FWB or HBOC-201. Secondarily, we assessed the feasibility of conversion from SAAP to ECLS to provide additional ongoing hemodynamic support. We hypothesized that SAAP followed by conversion to ECLS would be feasible and lead to reversal of lactic acidosis over the study period.

METHODS

Study Design and Overview

This study was approved by the Oregon Health and Science University Institutional Animal Care and Use Committee. Twenty-six fasting male Yorkshire swine (*Sus scrofa*, 78.8±3.9kg) were obtained from a single source animal vendor (Oakhill Genetics, Ewing, IL). Swine were randomly allocated into two groups (FWB and HBOC-201). Investigators were blinded to randomization during preparatory phases. The study protocol was divided into five phases: preparation, injury, intervention, damage control surgery (DCS) with balanced blood product resuscitation (DCR), and critical care (Figure 1).

After sedation, induction of anesthesia, preparation, and 10min stabilization, a liver injury was performed followed by controlled hemorrhage to induce HiTCA. Animals were then randomized and resuscitated in accordance with their intervention group. The pre-hospital phase consisted of 20min from SAAP intervention, followed by a 40min DCS and DCR phase, then a subsequent 260min critical care phase for a total study period of 320min.

Experimental Preparation

Sedation and analgesia were achieved with intramuscular Telazol (8mg/kg, Zoetis Services, Parsippany, NJ) and intramuscular buprenorphine (0.025mg/kg). Animals were intubated and anesthesia was maintained using continuous inhaled isoflurane (1-3%) to maintain general anesthesia until HiTCA was achieved. Tidal volumes were set at 6-8mL/kg, and end-tidal CO2 (EtCO2) was maintained in a range of 38-42mmHg until initiation of injury. The inspired fraction of oxygen was weaned to atmospheric level. After injury initiation, ventilator settings were not altered, allowing EtCO2 to be a surrogate for the effectiveness of resuscitation during the pre-hospital period.

Following induction, electrocardiographic monitoring was established. The left carotid artery, right femoral artery, right external jugular vein (EJV), left EJV (x2), and right femoral vein were cannulated using ultrasound-guided percutaneous Seldinger technique, with percutaneous catheters placed for blood sampling, physiologic monitoring, and subsequent resuscitation. A pulmonary artery thermodilution catheter (Edwards Lifesciences, Irvine, CA) was inserted through the right EJV sheath. A 14Fr sheath was inserted into the left femoral artery to facilitate deployment of the SAAP catheter. A right carotid artery cut down was performed to place an ultrasonic flow probe (Transonic Systems Inc, Ithaca, NY). A transesophageal defibrillation probe was fashioned by rolling the posterior external defibrillator pad (Zoll, Chelmsford, MA) around a 20Fr plastic tube stiffened by a flexible metal stylet. The anterior defibrillation pad was placed on the anterior left chest and the posterior pad was lubricated using electrically conductive gel and inserted into the esophagus so that the pad was situated posterior to the heart.

Surgical Preparation

In accordance with the established swine model of NCTH, a midline laparotomy and splenectomy were performed, four laparoscopic ports were placed through the abdominal wall, then the abdomen was closed with running suture and surgical staples.[27] The SAAP catheter was inserted into the left femoral sheath so that the deflated aortic balloon was positioned in the thoracic aorta. A 10-min stabilization period was observed prior to obtaining baseline blood samples and physiologic measurements.

Injury: Creation of NCTH and HiTCA

The abdomen was insufflated to a pressure of 15mmHg. The left lateral lobe of the liver was visualized then transected 3-4 cm away from the hilum using laparoscopic scissors, creating a

lethal grade-IV liver injury. The abdomen was then rapidly desufflated, all ports were removed, and the incisions were approximated using staples. After 5-minutes, a controlled hemorrhage was initiated at 1-2mL/kg/min until cardiac arrest was achieved. Cardiac arrest was defined as no pulsatile waveforms on the carotid arterial pressure tracing.

Intervention

At t=0, three minutes after onset of HiTCA, animals were randomly allocated to one of two groups: 1) SAAP with up to 6L of oxygenated FWB, or 2) SAAP with up to 6L of oxygenated HBOC-201. In both groups the intra-aortic balloon catheter (11.5Fr outer diameter, 7.5Fr inner diameter, 80cm long with a 17mL aortic occlusion balloon (Vention Medical Inc., Denver, CO)) was deployed so that the inflation of the balloon occluded Zone 1 of the aorta as defined by Morrison *et al.*[11] Immediately following balloon inflation, a 50mL bolus of lactated Ringer's solution was rapidly infused through the SAAP catheter to close the aortic valve, optimizing coronary artery perfusion and reducing left ventricular distension.[10]

a) FWB Group: Resuscitation with oxygenated FWB infused in conjunction with a 1% calcium chloride (CaCl) at a ratio of 8:1 to achieve a total infusion rate of 10mL/kg/min.[14] CaCl was required to chelate citrate in stored FWB and prevent ionized hypocalcemia, which was combined with FWB immediately prior to infusion.

b) HBOC-201 Group: HBOC-201 solution was prepared by dilution in lactated Ringer's solution to achieve an Hgb of 8.5g/dL. Resuscitation with oxygenated HBOC-201 was initiated at a rate of 10mL/kg/min.[23]

Selective Aortic Arch Perfusion Circuit

Bespoke SAAP circuits were constructed, consisting of a 3L reservoir (Belmont Instrument Corp., Billerica, MA), a Maquet Cardiohelp system and HLS-7 circuit (Maquet Inc., Wayne, NJ), and MASTERFLEX® peristaltic pumps (Cole-Parmer, Vernon Hills, IL). This apparatus included recirculation pathways and ports to convert the SAAP resuscitation into arterio-venous ECLS. (Supplemental Figure 1)

Pre-hospital resuscitation protocol and endpoints

SAAP resuscitation with either oxygenated FWB or HBOC-201 was maintained until ROSC was achieved. ROSC was defined as SBP>50mmHg with pulsatile carotid waveforms. If ROSC was not achieved by 2-min, or ventricular fibrillation developed, intra-aortic epinephrine (0.5mg) was administered every 30-sec (up to 2mg maximum), as needed, until ROSC. If ventricular fibrillation occurred, biphasic defibrillation (200J) was performed and repeated as necessary to achieve an organized electrocardiographic rhythm. Once ROSC was achieved, continuous inhaled isoflurane was restarted.

If following ROSC, the SBP dropped below 90mmHg or the MAP decreased below 50mmHg, a 250mL bolus of oxygenated FWB or HBOC-201 was administered via the SAAP catheter up to 2L. Boluses of FWB were given in conjunction with 1% CaCl, as previously described. No other interventions were performed during the pre-hospital phase.

Damage Control Surgery (DCS) Phase

After the 20-min pre-hospital phase, animals underwent simultaneous exploratory laparotomy and resuscitation with up to 3L of warmed intravenous FWB with concurrent 1% CaCl. The inspired fraction of oxygen was increased to 1.0. Resuscitation was titrated to SBP>90mmHg and MAP>60mmHg. Low ionized calcium (iCa) <0.9g/dL was treated with 1g 10% intravenous CaCl.

Profound acidosis (pH<7.10) was treated with 50mL of 8.4% sodium bicarbonate solution. Hyperkalemia (K^+>5.5meq/L) was treated with 10U of regular insulin and 50mL of 50% dextrose solution. Hypoglycemia (serum glucose <3mmol/L) was treated with 50mL of 50% dextrose solution.

DCS laparotomy was performed, with rapid evacuation of intra-abdominal shed blood and hemostasis of the liver injury using manual pressure and hemostatic clamps. Hemoperitoneum was collected and weighed. After clamping, the liver was packed with laparotomy sponges. The SAAP catheter balloon was deflated at a minimum time of 40min after initial injury (20-30min after initiation of SAAP intervention) to achieve standard ischemia times for intra-abdominal organs. Balloon deflation was only initiated after blood pressure resuscitation targets were met. Vasopressors were used in the setting of refractory hypotension with epinephrine injection or norepinephrine infusion used for concurrent SVR <80% of baseline and dobutamine infusion used for CO <80% of baseline despite fluid resuscitation.

Conversion to ECLS

A 15Fr venous ECMO catheter was placed in the right femoral vein and advanced to the inferior vena cava. Animals were heparinized to achieve an activated clotting time of twice the baseline level. ECLS was initiated at a flow rate of 500mL/min, which was the maximum flow rate achievable at 400mmHg given the resistance from the SAAP catheter and the intrinsic aortic pressure post-ROSC. A urinary catheter was inserted via cystotomy to track urine output. A temporary abdominal closure was performed using a sterile clear plastic cover secured over the bowel to minimize insensible fluid losses.[28]

Critical Care phase

After DCS and DCR, experimental treatment was continued using simulated critical care algorithms. Packed red blood cells (pRBC) (up to 6 units) were administered for Hgb<7g/dL. Fresh frozen plasma (FFP) (up to 6 units) was given for SBP<90mmHg with Hgb≥7g/dL. A norepinephrine infusion, up to 0.4mcg/kg/min was used to maintain SBP>90mmHg if animals were not fluid responsive and SVR <80% of baseline. A dobutamine infusion up to 20mcg/kg/min was used for inotropic support if the animal was not fluid responsive and CO <80% of baseline. FIO_2 was titrated down from 1.0 to maintain a normal pO2 based on ABGs. Intervention protocols for profound acidemia, hyperkalemia, hypocalcemia, and hypoglycemia were continued as described in the DCS section above. Active warming was achieved using a heated water-circulating blanket system. End of study was defined as 320-min post-SAAP intervention at which point animals were euthanized using sodium pentobarbital per institutional protocol. Following euthanasia, the liver was removed and weighed to quantify the liver transection. Total urine output (UOP) was recorded.

Data Acquisition

A data acquisition system was used to record high-frequency (500Hz) data for arterial blood pressure, EKG tracing, and carotid blood flow (BIOPAC Goleta, CA). Other variables were recorded at 60-sec intervals including heart rate (HR), ETCO2, central venous pressure (CVP), cardiac output (CO), SVR, stroke volume, central venous oxygen saturation (SvO2), and core temperature.

Arterial blood gas (pH, pO2, K+, iCa, lactate, base deficit) samples were obtained at baseline after a 10-min stabilization period, 5-min after SAAP intervention (t=5min), at arrival to hospital (t=20min), and every 30-min afterward until the end of the protocol.

Study Outcomes

Primary outcomes were rate of ROSC and end of experiment survival. Secondary outcomes included correction of hemodynamic derangements (HR, SBP, MAP, CO, SVR, carotid flow, SvO2, amount of vasopressor medications) and metabolic derangements (pH, lactate, base deficit, iCa, K+, Glucose) as well as resuscitation volumes (total volume of resuscitation during pre-hospital, DCS, and critical care phases of the experiment).

Statistical Analysis

Data were analyzed using SigmaPlot 12.0 (Systat Software, Inc, San Jose, California). Survival was analyzed with a log-rank test in conjunction with a Kaplan Meier survival plot. Categorical and continuous variables were analyzed using Fisher's exact test and two-tailed student's t-tests, respectively. Significance was defined as alpha <0.05.

RESULTS:

Baseline and Injury Characteristics

Baseline and injury characteristics are presented in Table 1. Physiologic and laboratory baseline values were similar between groups. There were no significant differences in liver injury parameters, or total blood loss per kg of body weight.

Induction of HiTCA

Cardiac arrest and resuscitation characteristics are presented in Table 2. Following injury, time to cardiac arrest was similar. During the 3-minute period of cardiac arrest, cardiac dysrhythmias

were common; asystole occurred in 25% of the FWB group versus 21% of the HBOC-201 group (p=1.000) and ventricular fibrillation occurred in 33% of the FWB group and 7% of the HBOC-201 group prior to intervention (p=0.148).

Aortic Hemostasis and Resuscitation using SAAP

Data collected in the SAAP intervention phase of the experiment is shown in Table 2. Time to initiation of SAAP therapy was similar between groups. ROSC was achieved in 100% of FWB animals vs 86% of HBOC-201 animals (p=0.483). The rate of ventricular fibrillation either before or immediately after initiation of SAAP was similar between groups (42% in FWB, 50% in HBOC-201). Two animals in the HBOC-201 group went into refractory ventricular fibrillation despite repeated defibrillation attempts and intra-aortic epinephrine. The time required to achieve ROSC was similar between groups. FWB animals required significantly more volume during initial SAAP resuscitation to achieve ROSC as compared to HBOC-201 (p=0.034), however required fewer additional post-ROSC bolus resuscitation during the remainder of the pre-hospital period (p=0.306) resulting in similar pre-hospital total fluid volumes administered between groups. We observed a rapid improvement in carotid blood pressure, HR, and carotid flow in both groups after initiating SAAP therapy (Figure 2). Carotid flow and heart rate in the FWB group were lower than the HBOC-201 group during the pre-hospital period. Carotid systolic and diastolic pressures were similar between groups. Pre-hospital survival was similar in both groups, with 100% survival for FWB and 86% with HBOC-201 (p=0.483). Both pre-hospital deaths in the HBOC-201 group occurred due to refractory ventricular fibrillation.

Damage Control Surgery and Resuscitation

DCS was performed in all animals that achieved ROSC. Two animals in the HBOC-201 group with initial successful ROSC were excluded during DCS due to equipment failure (1 due to calcium pump failure, 1 due to ECLS circuit failure). The single death in the FWB group was caused by vena caval thrombosis and pulmonary embolism; the two deaths in the HBOC-201 group occurred during DCS and were both secondary to profound hypotension refractory to fluid resuscitation and vasopressors. Time to definitive hemorrhage control and total volume of FWB resuscitation during DCS was similar between groups. All animals surviving DCS in both groups were successfully transitioned to ECLS after placement of a femoral venous catheter.

Critical Care Phase

Figure 3 depicts a Kaplan Meier survival curve for both experimental groups. Overall survival was not significantly different between groups, with 92% survival in the FWB group vs 67% in the HBOC-201 group (p=0.119).

Trends of serum lactate and pH over time are demonstrated in Figure 4. Serum lactate was significantly higher than baseline values at the end of the pre-hospital period in both groups, peaked at 80-min, then decreased over the critical care phase of the experiment. Serum lactate was similar between groups at baseline, 80-min (peak), and 320-min (end of experiment). In both groups, end of experiment lactate was significantly higher than the baseline level (10.1±8.2mmol/L vs 2.2±0.7mmol/L, p=0.008 FWB and 7.5±3.5mmol/L vs 1.9±0.5mmol/L, p=0.002 HBOC-201). Compared to 80-min (peak), lactate levels significantly improved by end of experiment in the HBOC-201 group (p=0.001) but not in the FWB group (p=0.104).

Serum pH significantly decreased after HiTCA and subsequent SAAP therapy, nadiring at 50-min. In both groups, pH significantly increased from 50-min to end of experiment. However, end of

experiment pH in both groups were significantly lower than baseline (7.31±0.16 vs 7.46±0.03 FWB and 7.31±0.09 vs 7.46±0.03, p=0.003 HBOC-201, p=0.007). There were no significant differences in pH between groups at baseline, 50-min, or 320-min. Similar amounts of 8.4% sodium bicarbonate were given in each group.

The amounts of fresh frozen plasma, norepinephrine, dobutamine, epinephrine, 50% dextrose, and insulin were similar between groups. Total urine output between groups was also similar between groups.

When comparing end of experiment physiologic and laboratory parameters between groups, there was a significant elevation in pulmonary artery pressure and carotid flow in the HBOC-201 group, compared to FWB (Table 1). In the HBOC-201 group, pulmonary artery pressures were significantly higher than baseline (41±6mmHg vs 30±5mmHg, p=0.021) and carotid flow was similar to baseline level (178±68mL/min vs 184±44mL/min, p=0.305). There were no other significant differences in end of experiment physiologic parameters between groups.

When comparing end of experiment parameters to baseline values within groups, there were significant differences in both the FWB and HBOC-201 animals (Table 1). HR was significantly increased in both groups and carotid SBP was significantly lower in both groups. Carotid blood flow was significantly lower in the FWB group but not the HBOC-201 group. CO was significantly decreased in the HBOC-201 group, but not the FWB group. SVR was significantly lower in both groups. There were no significant differences in temperature, hemoglobin or ionized calcium in either group.

Complications

There were two bleeding complications in the FWB group, both occurring after heparinization: one animal developed a retroperitoneal hematoma discovered on necropsy and a second had bleeding from the liver edge requiring re-laparotomy for hemorrhage control. There were three bleeding complications in the HBOC-201 group, all occurring after heparinization: one had re-bleeding from the liver edge requiring re-laparotomy for hemorrhage control, and two animals had abdominal wall bleeding requiring re-laparotomy. There was one thrombotic event in the FWB group prior to heparinization and conversion to ECLS with formation of fatal pulmonary embolism and thrombosis of the vena cava, which was confirmed on necropsy.

DISCUSSION:

SAAP is efficacious using either FWB or HBOC-201 in the reversal of HiTCA in the setting of NCTH in this experimental animal model. SAAP using both FWB and HBOC-201 achieved excellent rates of ROSC (100% vs 86%) and overall survival during a 320-minute study period (92% vs 67%). However, there were significant physiologic derangements from the ischemia perfusion injury that were still detectable after 5 hours.

Treatment for the range of hemorrhagic shock to HiTCA should also be managed by consideration of a continuum of support options for hemostasis and resuscitation (Supplemental Figure 2). After the balloon is inflated but without administration of fluids through the distal lumen, SAAP functions similarly to REBOA, which has been shown to be effective in the treatment of severe shock with less morbidity than resuscitative thoracotomy with aortic cross clamping[29].

However, SAAP allows for active intra-aortic resuscitation, which is more effective than passive aortic occlusion at achieving ROSC and 60-minute survival after HiTCA in a swine model[7]. The rates of ROSC and survival after 5 hours using SAAP in this study are significantly higher than

published rates of ROSC and survival after HiTCA in both swine and clinical studies using CPR[6], resuscitative thoracotomy[5, 7], or REBOA in conjunction with a balanced blood product resuscitation[8, 9, 13]. We also have demonstrated consistent efficacy of achieving ROSC using SAAP in animals with electrocardiographic asystole, which has been previously considered an unsalvageable condition[30].

SAAP is a promising new therapy in achieving ROSC and improving survival after HiTCA, but physiologic derangements caused by ischemia-reperfusion injury cannot be overlooked. The consequences of ischemia-reperfusion injury after balloon occlusion of the aorta are well-documented[31-34], and have led to development of strategies using partial[8, 32] or intermittent aortic occlusion[11]. However, partial occlusion of the aorta may decrease survival in the pre-hospital setting due to inadequate hemorrhage control[35]. Further work is needed to determine the optimum balance between hemostasis and ischemic sequelae in HiTCA, as previous studies have used less severe models of hemorrhagic shock.

In this study, we also demonstrated the ability to transition from aortic occlusion and resuscitation to ongoing cardiopulmonary support with veno-arterial ECLS using the SAAP catheter functioning as the arterial cannula, which only provided 500mL/min of support. A higher level of veno-arterial ECLS support may be helpful in the setting of severe physiologic derangements secondary to HiTCA and ischemia-reperfusion injury[25].

Additionally, the complexity of administration of intra-aortic oxygenated fresh whole blood in a pre-hospital environment must be considered as this technology is transitioned from the laboratory to the clinical setting. The therapeutic window of ionized calcium administration is small when given intra-aortic and even brief interruptions in concurrent calcium administration can lead to

ventricular fibrillation. HBOC-201's ability to be stored for up to 3 years at room temperature[36] and its iso-calcemic properties provide significant advantages in implementation of SAAP therapy, particularly in pre-hospital environments.

In this study, HBOC-201 appears to have similar rates of ROSC and overall survival as FWB similar physiologic derangements and logistically is much simpler to administer. The only significant variance in physiologic parameters between groups was pulmonary hypertension, which is a known side effect of HBOC-201, due to vasoconstriction and nitric oxide scavenging[37]. Previous studies have demonstrated that sodium nitrate may help counteract this pulmonary vasoconstriction in the setting of hemorrhagic shock[37]. HBOC-201 is currently only approved for clinical use in South Africa. However, it has been used in the United States for compassionate use in patients with severe anemia who refused blood products due to religious beliefs, showing an improvement in survival compared to conventional therapy[22]. Further work is needed to determine the efficacy of HBOC-201 versus standard therapy in pre-hospital settings where blood products are not available.

Complications

Ventricular fibrillation was a significant confounding factor in this study. Overall, ventricular fibrillation occurred in 67% of animals, including two episodes refractory to repeated defibrillation. Swine have a lower fibrillation threshold as compared to humans[38]. However, ventricular fibrillation in our model was more common than in previous studies utilizing SAAP therapy in a swine model[13, 14]. This could be swine model-related or reflect a reperfusion dysrhythmia event. Further studies are needed to determine the effect of SAAP therapy on ventricular fibrillation in this model of NCTH.

There were five bleeding complications, all occurring after full anticoagulation with heparin. While rate of bleeding events was similar between groups, further work is needed to determine the effects of HBOC-201 on the coagulation profile in the setting of hemorrhagic shock.

Limitations

Limitations of this study include its translational nature and the use of a swine model of NCTH though the choice of swine as a model was deliberate as swine cardiopulmonary and swine cardiovascular anatomy and physiology are similar to that of humans in response to traumatic injury. Physiologic monitoring used to guide clinical decisions may not be available in clinical settings, especially prior to arrival at a medical treatment facility. However, despite these limitations, the investigators are confident in the ability of the technology, as demonstrated, to be effective in future human trials.

CONCLUSION

SAAP utilizing either FWB or HBOC-201 is a promising therapy in the reversal of HiTCA, with significant survival advantages over existing therapies. This study demonstrates in an animal model that HiTCA may be reliably reversed by SAAP, but not without significant physiologic derangements associated with the subsequent ischemia-reperfusion. Further work is needed to determine the therapeutic window for aortic balloon occlusion in the setting of HiTCA, assess neurologic consequences, and streamline the steps needed to initiate SAAP therapy to improve safety and potential efficacy as human clinical trials are pursued.

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AUTHOR CONTRIBUTIONS:

Study design – JR, JM, HH, TG

Data Collection – HH, JM, TG, BM, SM, JR

Data Analysis and interpretation – HH, TG, JM, JR

Drafting Manuscript - HH

Critical revisions – HH, JM, TG, BM, SM, JR

REFERENCES

1. Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, Uribe P, Mallett O, Zubko T, Oetjen-Gerdes L, Rasmussen TE, Butler FK, Kotwal RS, Holcomb JB, Wade C, Champion H, Lawnick M, Moores L and Blackbourne LH. Death on the battlefield (2001-2011): implications for the future of combat casualty care. *J Trauma Acute Care Surg*. 2012;73:S431-7.

2. Chiara O, Scott JD, Cimbanassi S, Marini A, Zoia R, Rodriguez A, Scalea T and Milan Trauma Death Study G. Trauma deaths in an Italian urban area: an audit of pre-hospital and in-hospital trauma care. *Injury*. 2002;33:553-62.

3. Morrison JJ, Stannard A, Rasmussen TE, Jansen JO, Tai NR and Midwinter MJ. Injury pattern and mortality of noncompressible torso hemorrhage in UK combat casualties. *J Trauma Acute Care Surg*. 2013;75:S263-8.

4. Davis JS, Satahoo SS, Butler FK, Dermer H, Naranjo D, Julien K, Van Haren RM, Namias N, Blackbourne LH and Schulman CI. An analysis of prehospital deaths: Who can we save? *J Trauma Acute Care Surg*. 2014;77:213-8.

5. Cureton EL, Yeung LY, Kwan RO, Miraflor EJ, Sadjadi J, Price DD and Victorino GP. The heart of the matter: utility of ultrasound of cardiac activity during traumatic arrest. *J Trauma Acute Care Surg*. 2012;73:102-10.

6. Leis CC, Hernandez CC, Blanco MJ, Paterna PC, Hernandez Rde E and Torres EC. Traumatic cardiac arrest: should advanced life support be initiated? *J Trauma Acute Care Surg*. 2013;74:634-8.

7. Barnard E, Yates D, Edwards A, Fragoso-Iniguez M, Jenks T and Smith JE. Epidemiology and aetiology of traumatic cardiac arrest in England and Wales - A retrospective database analysis. *Resuscitation*. 2017;110:90-94.

8. Matsumura Y, Matsumoto J, Kondo H, Idoguchi K, Funabiki T and investigators D-I. Partial occlusion, conversion from thoracotomy, undelayed but shorter occlusion: resuscitative endovascular balloon occlusion of the aorta strategy in Japan. *Eur J Emerg Med*. 2017.

9. Norii T, Crandall C and Terasaka Y. Survival of severe blunt trauma patients treated with resuscitative endovascular balloon occlusion of the aorta compared with propensity score-adjusted untreated patients. *J Trauma Acute Care Surg*. 2015;78:721-8.

10. Manning JE, Murphy CA, Jr., Hertz CM, Perretta SG, Mueller RA and Norfleet EA. Selective aortic arch perfusion during cardiac arrest: a new resuscitation technique. *Ann Emerg Med*. 1992;21:1058-65.

11. Morrison JJ, Ross JD, Houston Rt, Watson JD, Sokol KK and Rasmussen TE. Use of resuscitative endovascular balloon occlusion of the aorta in a highly lethal model of noncompressible torso hemorrhage. *Shock*. 2014;41:130-7.

12. Brenner ML, Moore LJ, DuBose JJ, Tyson GH, McNutt MK, Albarado RP, Holcomb JB, Scalea TM and Rasmussen TE. A clinical series of resuscitative endovascular balloon occlusion of the aorta for hemorrhage control and resuscitation. *J Trauma Acute Care Surg*. 2013;75:506-11.

13. Barnard EBG MJ, Smith JE, Rall JM, Cox JM, Ross JD. A comparison of Selective Aortic Arch Perfusion and Resuscitative Endovascular Balloon Occlusion of the Aorta for the management of hemorrhage-induced traumatic cardiac arrest: A translational model in large swine. *PLoS Med*. 2017.

14. Manning JE, Ross JD, McCurdy SL and True NA. Aortic Hemostasis and Resuscitation: Preliminary Experiments Using Selective Aortic Arch Perfusion With Oxygenated Blood and Intra-aortic Calcium Coadministration in a Model of Hemorrhage-induced Traumatic Cardiac Arrest. *Acad Emerg Med*. 2016;23:208-12.

15. Manning JE. Feasibility of blind aortic catheter placement in the prehospital environment to guide resuscitation in cardiac arrest. *J Trauma Acute Care Surg*. 2013;75:S173-7.

16. Sadek S, Lockey DJ, Lendrum RA, Perkins Z, Price J and Davies GE. Resuscitative endovascular balloon occlusion of the aorta (REBOA) in the pre-hospital setting: An additional resuscitation option for uncontrolled catastrophic haemorrhage. *Resuscitation*. 2016;107:135-8.

17. Morrison JJ, Galgon RE, Jansen JO, Cannon JW, Rasmussen TE and Eliason JL. A systematic review of the use of resuscitative endovascular balloon occlusion of the aorta in the management of hemorrhagic shock. *J Trauma Acute Care Surg*. 2016;80:324-34.

18. Natanson C, Kern SJ, Lurie P, Banks SM and Wolfe SM. Cell-free hemoglobin-based blood substitutes and risk of myocardial infarction and death: a meta-analysis. *Jama*. 2008;299:2304-12.

19. Jahr JS, Mackenzie C, Pearce LB, Pitman A and Greenburg AG. HBOC-201 as an alternative to blood transfusion: efficacy and safety evaluation in a multicenter phase III trial in elective orthopedic surgery. *J Trauma*. 2008;64:1484-97.

20. Van Hemelrijck J, Levien LJ, Veeckman L, Pitman A, Zafirelis Z and Standl T. A safety and efficacy evaluation of hemoglobin-based oxygen carrier HBOC-201 in a randomized, multicenter red blood cell controlled trial in noncardiac surgery patients. *Anesth Analg*. 2014;119:766-76.

21. Auker CR and McCarron RM. US Navy experience with research on, and development of, hemoglobin-based oxygen carriers. *J Trauma*. 2011;70:S40-1.

22. Weiskopf RB, Beliaev AM, Shander A, Guinn NR, Cap AP, Ness PM and Silverman TA. Addressing the unmet need of life-threatening anemia with hemoglobin-based oxygen carriers. *Transfusion*. 2017;57:207-214.

23. Manning JE, Katz LM, Pearce LB, Batson DN, McCurdy SL, Gawryl MS and Baker CC. Selective aortic arch perfusion with hemoglobin-based oxygen carrier-201 for resuscitation from exsanguinating cardiac arrest in swine. *Crit Care Med*. 2001;29:2067-74.

24. Burke CR, Crown A, Chan T and McMullan DM. Extracorporeal life support is safe in trauma patients. *Injury*. 2017;48:121-126.

25. Tseng YH, Wu TI, Liu YC, Lin PJ and Wu MY. Venoarterial extracorporeal life support in post-traumatic shock and cardiac arrest: lessons learned. *Scand J Trauma Resusc Emerg Med*. 2014;22:12.

26. Bedeir K, Seethala R and Kelly E. Extracorporeal life support in trauma: Worth the risks? A systematic review of published series. *J Trauma Acute Care Surg*. 2017;82:400-406.

27. Ross JD, Burns CJ, Sagini EM, Zarzabal LA and Morrison JJ. A laparoscopic swine model of noncompressible torso hemorrhage. *J Trauma Acute Care Surg*. 2014;77:S77-82.

28. Fernandez L, Norwood S, Roettger R and Wilkins HE, 3rd. Temporary intravenous bag silo closure in severe abdominal trauma. *J Trauma*. 1996;40:258-60.

29. White JM, Cannon JW, Stannard A, Markov NP, Spencer JR and Rasmussen TE. Endovascular balloon occlusion of the aorta is superior to resuscitative thoracotomy with aortic clamping in a porcine model of hemorrhagic shock. *Surgery*. 2011;150:400-9.

30. Millin MG, Galvagno SM, Khandker SR, Malki A, Bulger EM, Standards, Clinical Practice Committee of the National Association of EMSP and Subcommittee on Emergency Services-Prehospital of the American College of Surgeons' Committee on T. Withholding and termination of resuscitation of adult cardiopulmonary arrest secondary to trauma: resource document to the joint NAEMSP-ACSCOT position statements. *J Trauma Acute Care Surg*. 2013;75:459-67.

31. Morrison JJ, Ross JD, Markov NP, Scott DJ, Spencer JR and Rasmussen TE. The inflammatory sequelae of aortic balloon occlusion in hemorrhagic shock. *The Journal of surgical research*. 2014;191:423-31.

32. Russo RM, Neff LP, Lamb CM, Cannon JW, Galante JM, Clement NF, Grayson JK and Williams TK. Partial Resuscitative Endovascular Balloon Occlusion of the Aorta in Swine Model of Hemorrhagic Shock. *Journal of the American College of Surgeons*. 2016;223:359-68.

33. Williams TK, Neff LP, Johnson MA, Ferencz SA, Davidson AJ, Russo RM and Rasmussen TE. Extending resuscitative endovascular balloon occlusion of the aorta: Endovascular variable aortic control in a lethal model of hemorrhagic shock. *J Trauma Acute Care Surg*. 2016;81:294-301.

34. Avaro JP, Mardelle V, Roch A, Gil C, de Biasi C, Oliver M, Fusai T and Thomas P. Forty-minute endovascular aortic occlusion increases survival in an experimental model of uncontrolled hemorrhagic shock caused by abdominal trauma. *J Trauma*. 2011;71:720-5; discussion 725-6.

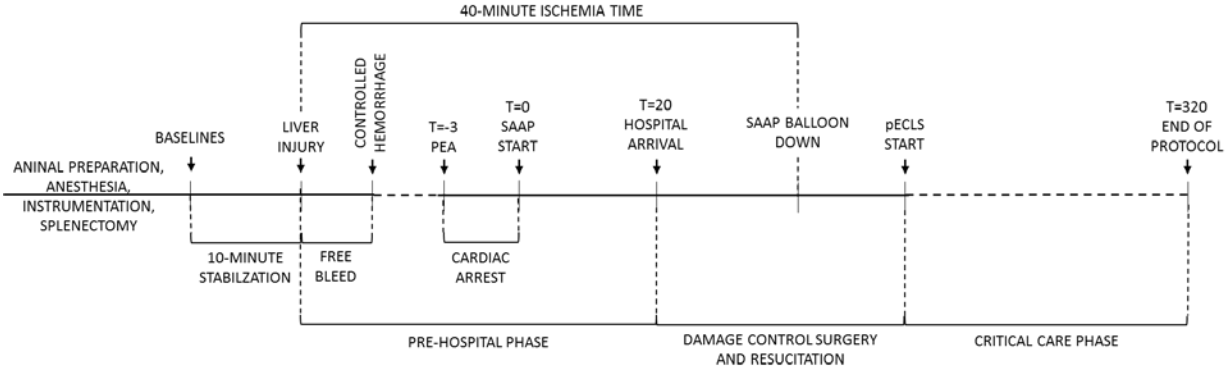
35. Russo RM, Williams TK, Grayson JK, Lamb CM, Cannon JW, Clement NF, Galante JM and Neff LP. Extending the golden hour: Partial resuscitative endovascular balloon occlusion of the aorta in a highly lethal swine liver injury model. *J Trauma Acute Care Surg*. 2016;80:372-8; discussion 378-80.

36. Meyerson SL, Sternbach JM, Zwischenberger JB and Bender EM. The Effect of Gender on Resident Autonomy in the Operating room. *J Surg Educ*. 2017.

37. Moon-Massat P, Scultetus A, Arnaud F, Brown A, Haque A, Saha B, Kim B, Sagini E, McGwin G, Jr., Auker C, McCarron R and Freilich D. The effect HBOC-201 and sodium nitrite resuscitation after uncontrolled haemorrhagic shock in swine. *Injury*. 2012;43:638-47.

38. Walcott GP, Kroll MW and Ideker RE. Ventricular fibrillation: are swine a sensitive species? *Journal of interventional cardiac electrophysiology : an international journal of arrhythmias and pacing*. 2015;42:83-9.

Figure 1 – Study protocol timeline



PEA – pulseless electrical activity; SAAP – Selective Aortic Arch Perfusion; ECLS – Extracorporeal Life Support

Figure 2 – Secondary outcomes – Averaged (a) carotid artery systolic and diastolic blood pressure, (b) femoral systolic pressure, (c) left carotid artery flow, (d) heart rate, (e) pulmonary artery mean arterial pressure, (f) systemic vascular resistance, (g) cardiac output, (h) end-tidal carbon dioxide.

SAAP – Selective Aortic Arch Perfusion; FWB – Fresh whole blood; HBOC – Hemoglobin-based oxygen carrier.

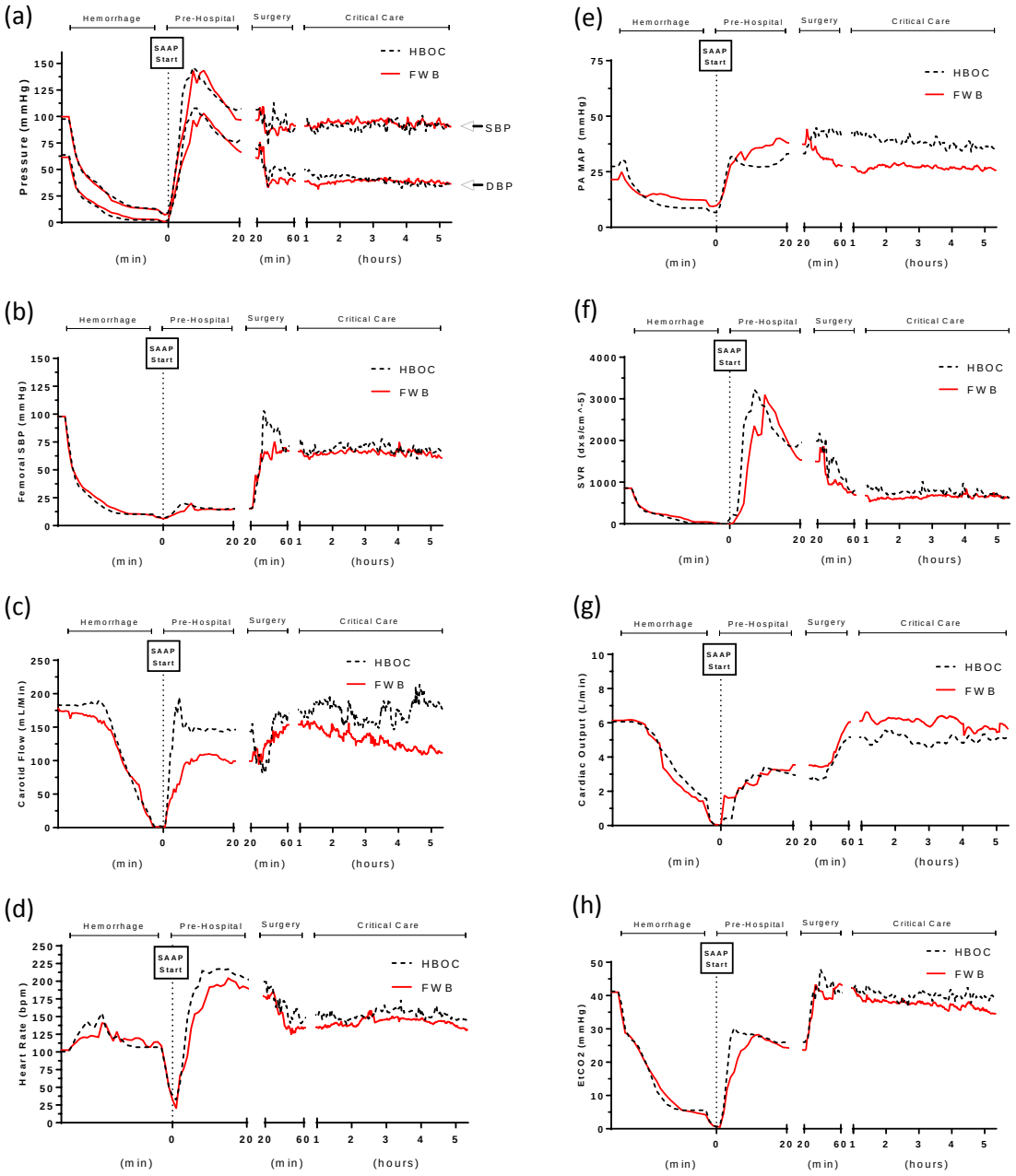
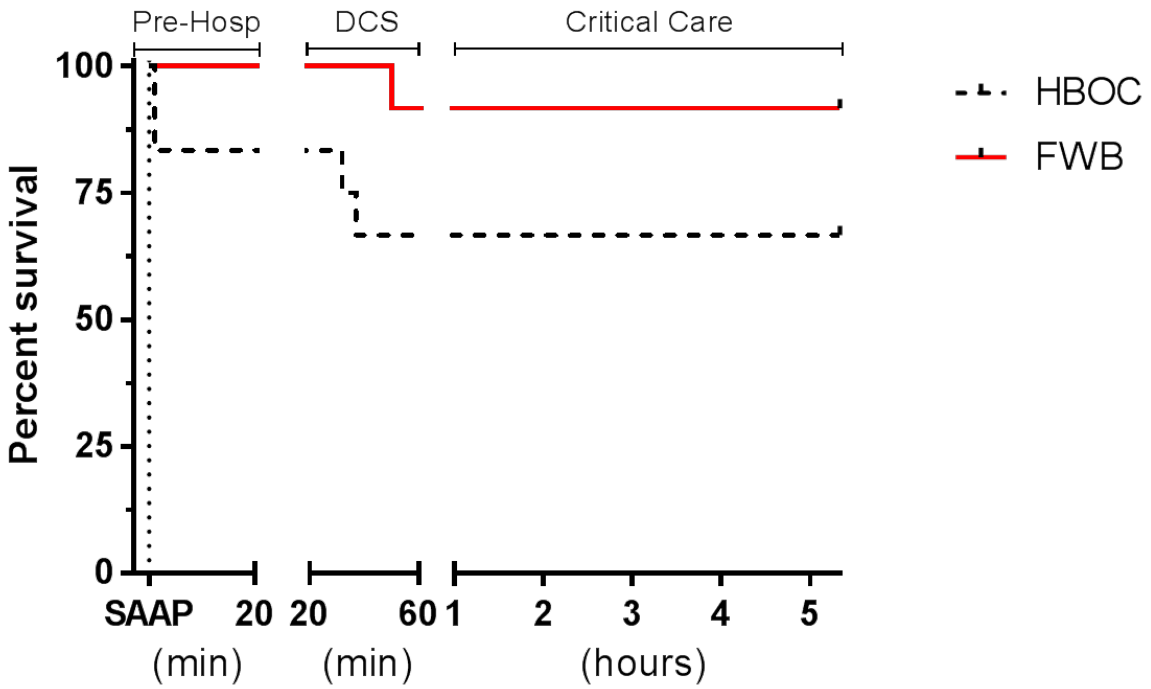
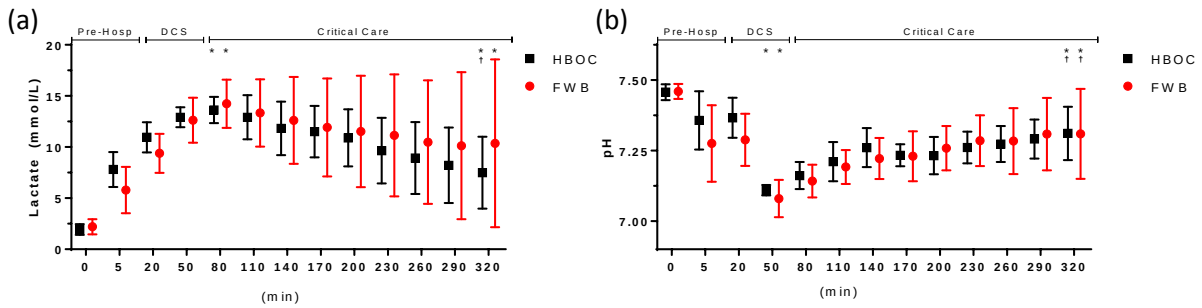


Figure 3 - Primary outcome – Overall (320 min) survival of HBOC group (n=14), compared to FWB group (n=12)



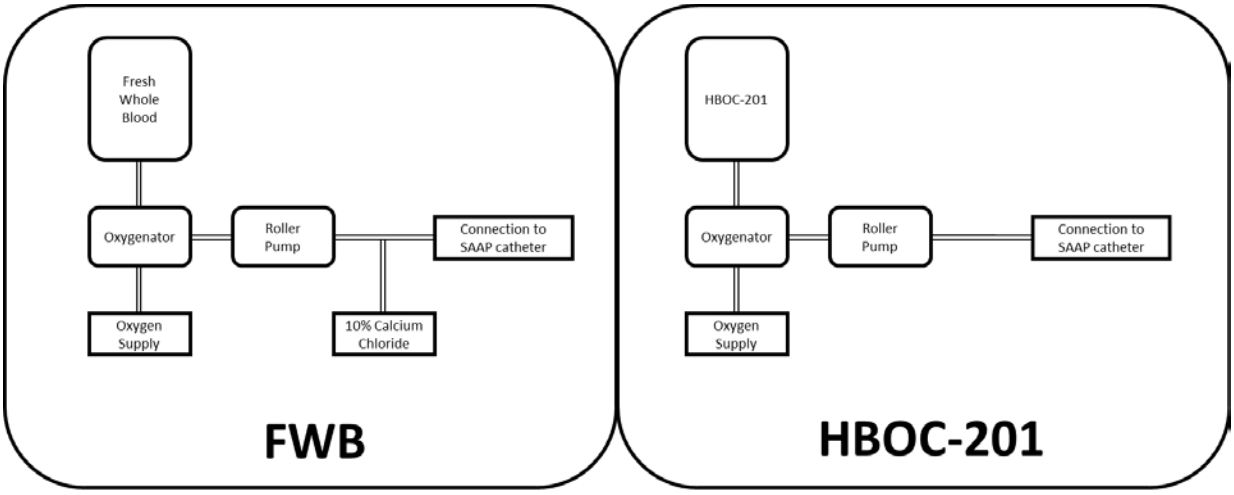
SAAP – Selective Aortic Arch Perfusion; FWB – Fresh whole blood; HBOC – Hemoglobin-based oxygen carrier; DCS – Damage Control Surgery

Figure 4 – Metabolic changes in response to hemorrhage-induced traumatic cardiac arrest and balloon occlusion of the aorta (a) trend in serum lactate over time; (b) trend in serum pH over time



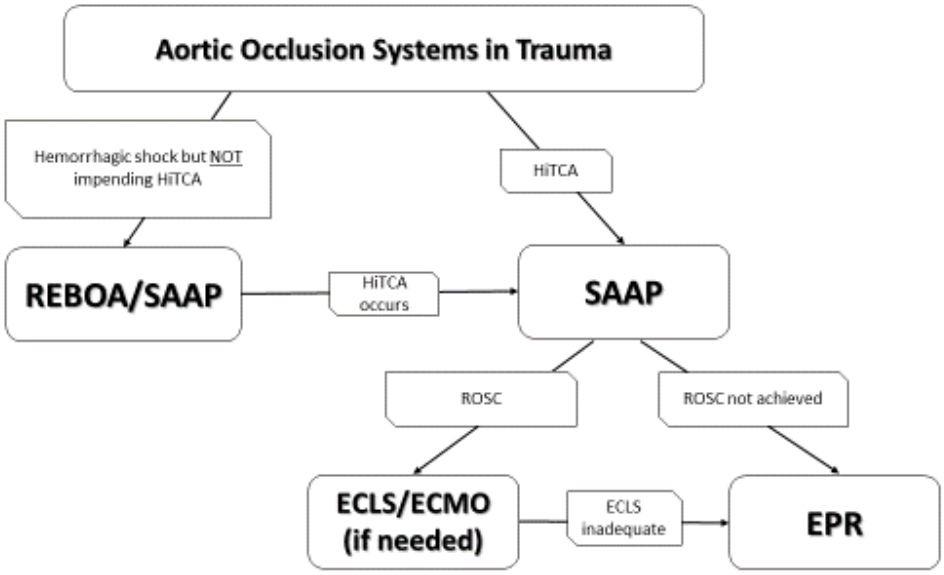
SAAP – Selective Aortic Arch Perfusion; FWB – Fresh whole blood; HBOC – Hemoglobin-based oxygen carrier; * represents a statistically significant difference from baseline; † represents a statistically significant difference from peak or nadir.

Supplemental Figure 1 – Bespoke SAAP circuit diagrams for each experimental group



SAAP – Selective Aortic Arch Perfusion; FWB – Fresh Whole Blood; HBOC – Hemoglobin-based oxygen carrier. During SAAP therapy, fresh whole blood or HBOC-201 is stored in a 3L reservoir, then oxygenated and pumped into the aorta in a retrograde fashion. When using fresh whole blood, concurrent administration of calcium chloride solution is necessary to maintain a normal range of ionized calcium.

Supplemental Figure 2 – Spectrum of aortic interventions in the treatment of hemorrhagic shock and hemorrhage-induced cardiac arrest



REBOA – Resuscitative Endovascular Balloon Occlusion of the Aorta; SAAP – Selective Aortic Arch Perfusion; HiTCA – Hemorrhage-induced Traumatic Cardiac Arrest; ECLS – Extracorporeal Life Support; ROSC – Return of spontaneous circulation; EPR – Emergency Preservation and Resuscitation

Table 1: Baseline, Injury, and End Experiment Characteristics

Parameter	Baseline			End of Experiment		
Preparation	*FWB (n = 12)*	*HBOC-201 (n = 14)*	*p-value*			
Weight, kg	77.4 ± 3.7	80.0 ± 3.7	0.094			
Spleen weight, g	719 ± 146	758 ± 150	0.509			
Total preparation time, min	134 ± 31	138 ± 32	0.738			
Physiologic parameters	*FWB (n = 12)*	*HBOC-201 (n = 14)*	*p-value*	*FWB (n = 11)*	*HBOC-201 (n = 8)*	*p-value*
Heart rate, beats/minute	103 ± 26	101 ± 18	0.827	131 ± 23†	145 ± 26†	0.243
Systemic SBP, mmHg	99.8 ± 8.6	97.6 ± 9.5	0.533	90.4 ± 15.1†	88.3 ± 10.1†	0.736
Femoral SBP, mmHg	97.9 ± 10.8	97.8 ± 15.9	0.981	60.6 ± 18.8†	65.1 ± 22.7†	0.643
Pulmonary SBP, mmHg	26.9 ± 6.6	29.8 ± 5.3	0.232	29.9 ± 10.6	40.6 ± 6.32†	0.021*
Carotid flow, mL/minute	190 ± 31	184 ± 44	0.685	111 ± 57†	178 ± 68	0.033*
CVP, mmHg	11.5 ± 1.8	11.9 ± 2.5	0.689	10.3 ± 1.9	9.6 ± 2.4	0.513
CO, L/min	6.13 ± 1.07	6.07 ± 0.84	0.871	5.66 ± 1.29	5.18 ± 0.88†	0.391
SVR, dyn·s/cm	854 ± 173	858 ± 184	0.953	623 ± 191†	625 ± 175†	0.985
EtCO2, mmHg	41.0 ± 2.6	41.3 ± 2.6	0.781	34.5 ± 7.2†	39.1 ± 3.8	0.119
Temperature, C	38.2 ± 0.9	38.1 ± 1.4	0.963	37.2 ± 3.4	38.5 ± 1.6	0.335
pH	7.46 ± 0.03	7.46 ± 0.03	0.805	7.31 ± 0.16†	7.31 ± 0.10†	0.980
pO2, mmHg	99.4 ± 15.5	103.5 ± 19.3	0.559	250.2 ± 70.3†	239.1 ± 66.2†	0.733
pCO2, mmHg	41.1 ± 2.0	41.1 ± 2.1	0.958	39.8 ± 6.2	45.0 ± 6.7	0.099
Hgb, g/dL	10.3 ± 0.9	10.1 ± 0.8	0.534	9.5 ± 1.3	9.4 ± 0.8	0.935
Potassium, mmol/L	4.0 ± 0.2	4.1 ± 0.3	0.568	5.1 ± 0.8†	5.1 ± 0.7†	1.000
Ionized calcium, mmol/L	1.32 ± 0.04	1.30 ± 0.04	0.188	1.28 ± 0.06	1.31 ± 0.08	0.388
Lactate, mmol/L	2.2 ± 0.8	1.9 ± 0.5	0.290	10.4 ± 8.2†	7.5 ± 3.5†	0.367
Base deficit	5.4 ± 1.8	5.2 ± 2.4	0.845	-4.9 ± 9.7†	-3.5 ± 5.2†	0.711
Serum glucose, mmol/L	5.0 ± 1.2	5.5 ± 0.9	0.194	4.8 ± 2.1	4.3 ± 2.1	0.669
Injury Characteristics	*FWB (n = 12)*	*HBOC-201 (n = 14)*	*p-value*			
Transection duration, sec	58.8 ± 20.5	58.9 ± 17.1	0.997			
Time to HiTCA, sec	862 ± 350	816 ± 166	0.66			
Resected lobe, %	65.9 ± 10.8	70.2 ± 6.0	0.24			
Controlled hemorrhage, mL	794 ± 542	778 ± 311	0.934			
Hemoperitoneum, mL	3450 ± 737	2990 ± 297	0.08			
Total blood loss, mL/kg	54.9 ± 14.1	46.8 ± 6.4	0.107			
Cardiac rhythm immediately prior to intervention	*FWB (n = 12)*	*HBOC-201 (n = 14)*	*p-value*			
Perfusable rhythm, n (%)	4 (33)	6 (43)	0.701			
Ventricular fibrillation, n (%)	4 (33)	1 (7)	0.148			
Agonal, n (%)	1 (8)	4 (29)	0.330			
Asystole, n (%)	3 (25)	3 (21)	1.000			

Note: "*" denotes statistically significant difference between FWB and HBOC-201 groups. "†" denotes statistically significant difference between end of experiment and baseline parameter within the experimental group.

Table 2: SAAP therapy, DCS, DCR, and ICU

Parameter	FWB	HBOC-201	Statistical test
SAAP Intervention	*FWB (n = 12)*	*HBOC-201 (n = 14)*	*p-value*
Perfusate pO2, mmHg	730 ± 48	788 ± 44	0.004*
Perfusate Hgb, g/dL	8.8 ± 0.6	8.7 ± 0.2	0.483
Perfusate potassium, mmol/L	6.3 ± 1.3	4.3 ± 0.21	<0.001
Perfusate calcium, mmol/L	0.07 ± 0.02	1.03 ± 0.06	<0.001
Time from HiTCA to SAAP, seconds	183 ± 2.27	182 ± 1.05	0.111
Time to ROSC, seconds	262 ± 217	133 ± 89.3	0.069
SAAP volume to ROSC, mL	2180 ± 1060	1430 ± 469	0.034*
SAAP bolus, mL	83 ± 163	177 ± 264	0.306
Total SAAP volume, mL	2260 ± 1140	1600 ± 480	0.079
ROSC, n (%)	12 (100)	12 (86)	0.483
Damage Control Surgery and Resucitation	*FWB (n = 11)*	*HBOC-201 (n = 8)*	*p-value*
Time to definitive control, minutes	8.88 ± 1.59	8.98 ± 2.74	0.930
Ischemia time, minutes	43.6 ± 3.3	42.5 ± 1.8	0.408
Need for relaparotomy, n (%)	1 (9)	2 (25)	0.586
DCS fresh whole blood, mL	2974 ± 617	3271 ± 505	0.254
Intensive Care Phase	*FWB (n = 11)*	*HBOC-201 (n = 8)*	*p-value*
Fresh frozen plasma, mL	1659 ± 392	1500 ± 231	0.321
Norepinephrine, mg	3.9 ± 3.2	5.2 ± 3.7	0.422
Dobutamine, mg	30.8 ± 66.4	57.4 ± 60.8	0.384
Epinephrine, mg	0.8 ± 0.8	0.6 ± 0.5	0.599
50% Glucose, mL	104.5 ± 68.8	100.8 ± 54.3	0.899
8.4% Sodium Bicarbonate, mL	63.7 ± 80.9	31.3 ± 25.9	0.293
Insulin, units	10.9 ± 0.7	8.9 ± 0.6	0.503
Urine output, mL	189 ± 234	96 ± 112	0.297
Complications	*FWB (n = 12)*	*HBOC-201 (n = 12)*	*p-value*
Bleeding, n (%)	2 (17)	3 (21)	1.000
Thombosis, n (%)	1(8)	0(0)	1.000
Death, n (%)	1(8)	4(33)	0.317

Note: “*” denotes a statistically significant difference between the FWB and HBOC-201 experimental groups.