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A hemoglobin-based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) versus hetastarch (HEX) in a moderate severity hemorrhagic shock swine model with delayed evacuation☆

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Abstract

Objective: To evaluate the efficacy of HBOC-201 for resuscitation of hemorrhagic shock in a swine model incorporating soft tissue injury and delayed evacuation.
Methods: A muscle crush injury and 40% estimated blood volume controlled hemorrhage was completed in 24 Yucatan mini-pigs. Pigs were untreated or resuscitated with HBOC-201 or 6% hetastarch (HEX) at 20 min. Invasive hemodynamics and clinical variables were monitored for 4 h (pre-hospital phase) and subsequent fluid infusions were administered for severe hypotension or tachycardia. Animals were recovered from anesthesia and monitored non-invasively to 72 h (hospital phase).
Results: 100% (8/8) of HBOC-201-, 88% (7/8) of HEX-, and 63% (5/8) of non-resuscitated pigs, survived to 72 h ($p = 0.27$). Mean arterial pressure, mean pulmonary arterial pressure and systemic vascular resistance index were higher in HBOC-201 pigs. By 90 min, cardiac index was restored to baseline in the HBOC-201 group and was 1.4-fold greater than baseline in the HEX group. HBOC-201 pigs had lower fluid requirements than HEX pigs (18.8 ± 1.8 and 29.9 ± 1.1 ml/kg, $p < 0.001$) in the pre-hospital phase and required fewer blood transfusions (1.3 ± 1.3 and 9.4 ± 0.6 ml/kg, respectively, $p < 0.001$) in the hospital phase. Urine output and blood creatinine were comparable in HBOC-201 and HEX pigs. Tissue oxygenation levels were highest in the HBOC-201 group.
Conclusions: As HBOC-201 restored hemodynamics and tissue oxygenation and decreased fluid requirements, in comparison with HEX, HBOC-201 was at least as efficacious and possibly a superior resuscitative fluid in a military-relevant delayed evacuation hemorrhagic shock swine model.
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Keywords: Fluid therapy; Hemorrhage; Hypovolemia; Resuscitation; Shock; Trauma

1. Introduction

Investigation of hemoglobin-based oxygen carriers (HBOC) for use as primary resuscitation fluids for hemorrhagic shock (HS) causalities in the pre-hospital setting, where blood is not available (rural trauma or military operations), has recently gained prominence in trauma research [1–4]. HBOC are chemically modified hemoglobin solutions

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(e.g., polymerized or conjugated) that are universally compatible. HBOC can be heat-treated, and therefore the risk of transmission of communicable pathogens is low. A bovine polymerized HBOC (HBOC-201) is room temperature stable with a long shelf-life (∼3 years). HBOC offer benefits over standard intravenous (IV) fluids (i.e., crystalloids or colloids) because they have oxygen carrying as well as volume expansion capacity. In animal models of HS, HBOC are efficacious at low volumes, thereby decreasing logistical constraints, resuscitation-related hemodilution, fluid overload, and consequent complications [5,6]. They can be administered easily by simple IV administration without special training. Furthermore, some HBOC have been evaluated in phase I, II, and III clinical trials for trauma and non-trauma uses [7–10].

In the austere environment of military combat, prolonged evacuation times, logistical constraints, as well as the devastating nature of combat injuries frequently present extreme challenges to pre-hospital medical providers. Research evaluating HBOC as resuscitation fluids for military combat HS casualties has generated significant interest recently. The use of HBOC as low volume, universally compatible, and oxygen carrying resuscitative fluids could significantly improve pre-hospital care of HS casualties.

The aims of the present study were to: 1. develop a military relevant pre-hospital swine HS model incorporating controlled hemorrhage, soft tissue injury, limited resuscitation volume, and delayed arrival to definitive care and 2. use this model to test our hypotheses that resuscitation with HBOC-201 would stabilize hemodynamics, decrease fluid requirements, improve key surrogates of tissue perfusion, and decrease morbidity and mortality, in comparison with the standard resuscitative fluid carried by *US Special Operations Forces*, 6% hetastarch in LR (Hextend®).

2. Materials and methods

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. The study was approved by the Naval Medical Research Center (NMRC)/Walter Reed Army Institute of Research (WRAIR) Institutional Animal Care and Use Committee (IACUC) and all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International (AAALAC).

2.1. Animal preparation

Twenty-four male and female Yucatan Mini pigs (∼26 kg) (Sinclair Research Center, Inc., Columbia, MO) were used. Feed and water were withheld 12–14 h prior to initiation of the experiment. Animals were sedated and anesthesia induced with intramuscular ketamine hydrochloride (33 mg/kg) and atropine sulfate (0.05 mg/kg), followed by mask ventilation with isoflurane (3.0%) with FiO_2 1.0, to facilitate tracheal intubation. Anesthesia was maintained via isoflurane (1–2.5%) with FiO_2 0.21. Animals were allowed to breathe spontaneously throughout the experiment. Anesthesia-induced apnea was addressed with ventilatory support (Ohmeda 7800 series ventilator, Datex, Madison, WI) at 12–15 breaths/min and tidal volume 5–10 ml/kg. No significant differences were observed in pCO_2 or pH prior to hemorrhage. Body temperature was monitored and maintained at $99 \pm 1\,^{\circ}F$ with a thermal blanket. Bladder catheterization for urine collection was accomplished in females via insertion of a Foley catheter, directly into the urethra and in males via laproatomy and direct bladder catheterization. The right external jugular vein and carotid artery were dissected and isolated. A 9 F introducer sheath was placed in the external jugular vein using Seldinger technique and a 7.5 F pulmonary artery catheter (PAC; Edwards Life Sciences, Irvine, CA,) was inserted for continuous hemodynamic and cardiac output (CO) monitoring. A 20 G angiocatheter was placed in the carotid artery and mean arterial pressure (MAP) and heart rate (HR) were continuously monitored. A 3–5 cm lower abdominal incision was made and the left *rectus abdominus* muscle located. The rectus sheath was mobilized bluntly and a surgical tissue clamp (Kocher) placed on a standardized portion of the muscle in the center of the incision. All surgical procedures were performed using aseptic techniques. Blood volume (ml) was estimated as: EBV = animal weight (kg) × 65 ml/kg.

2.2. Pre-hospital phase: tissue injury, hemorrhage and resuscitation

The Kocher clamp was closed for 5 min to crush the portion of the rectus abdominus muscle to create a soft tissue injury, and pigs were bled by 40% of their EBV via the external jugular vein and/or the carotid artery over 15 min to induce HS (Fig. 1). The blood volume was withdrawn at a rate that decreased over time in a stepwise fashion with half of the target hemorrhage volume occurring in the first 5 min and the remaining volume over the next 10 min. All "shed" blood was collected in sterile blood bags containing citrate phosphate dextrose (CPDA-1, Fenwal, Baxter, Deerfield, IL) for possible later re-infusion. Pigs were allocated randomly to one of three treatment groups: hemoglobin-based oxygen carrier (HBOC, HBOC-201, Hemopure®, Biopure Corp., Cambridge, MA); 6% hetastarch in LR (HEX, Hextend®, Abbott Laboratories, Abbot Park, IL); or no fluids (NON). At 20 min, resuscitated pigs were administered 10 ml/kg of HBOC (equivalent to ∼1/2 the Hb load of one unit of PRBCs) or HEX over 10 min. Additional infusions of 5 ml/kg were provided at 30, 60, 120, and 180 min post-injury if hypotension (MAP < 60 mmHg) or tachycardia (HR > baseline value [time 0]) were observed. Fluids were infused at room temperature.