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Original Contribution

Resuscitation from hemorrhagic shock using polymerized hemoglobin compared to blood☆,☆☆

Daniel Ortiz, MSc*, Marcelo Barros, BSc, Su Yan, PhD, Pedro Cabrales, PhD

Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093-0412, USA

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ABSTRACT

The development of an alternative to blood transfusion to treat severe hemorrhage remains a challenge, especially in far forward scenarios when blood is not available. Hemoglobin level (Hb)–based oxygen (O_2) carriers (HBOCs) were developed to address this need. Hemopure (HBOC-201, bovine Hb glutamer-250; OPK Biotech, Cambridge, MA), one such HBOC, has been approved for clinical use in South Africa and Russia. At the time of its approval, however, few studies aimed to understand Hemopure's function, administration, and adverse effects compared to blood. We used intravital microscopy to study the microcirculation hemodynamics (arteriolar and venular diameters and blood flow and functional capillary density [FCD]) and oxygenation implications of Hemopure administration at different Hb concentrations—4, 8, and 12 gHb/dL—compared to fresh blood transfusion during resuscitation from hemorrhagic shock. Experiments were performed in unanesthetized hamsters instrumented with a skinfold window chamber, subjected to hemorrhage (50% of the blood volume), followed by 1-hour hypovolemic shock and fluid resuscitation (50% of the shed volume). Our results show that fluid resuscitation with Hemopure or blood restored systemic and microvascular parameters. Microcirculation O_2 delivery was directly correlated with Hemopure concentration, although increased vasoconstriction was as well. Functional capillary density reflected the balance between enhanced O_2 transport and reduced blood flow: 12 gHb/dL of Hemopure and blood decreased FCD compared to the lower concentrations of Hemopure ($P < .05$). The balance between O_2 transport and tissue perfusion can provide superior resuscitation from hemorrhagic shock compared to blood transfusion by using a low Hb concentration of HBOCs relative to blood.

1. Background

In the United States, allogeneic red blood cell (RBC) transfusion has long been considered an important treatment option for patients with blood losses [1]. However, the supply of blood is particularly vulnerable to pandemics [2,3], and blood transfusion-related adverse events, both short and long term, are among the costliest contributor to health care expenditures [4]. In addition, the availability of blood is limited in emergency situations such as in war zones or after natural disasters [5]. Therefore, the development of a therapeutic product that can suitably replace blood transfusion in cases of severe hemorrhage or during major cardiovascular surgery has been a goal of scientific and commercial efforts [6]. Hemoglobin (Hb), the protein responsible for the transport of O_2 in the RBC, has served as the precursor for the formulation of blood substitutes. Hemoglobin-based oxygen (O_2) carriers (HBOCs) were developed to restore intravascular volume and O_2-carrying capacity [7]. Previous HBOCs were based on stroma-free Hb [8,9], the tetrameter structure of which would dissociate upon infusion, producing several adverse reactions including kidney failure [10,11].

Those that produced the first generation of HBOCs tried to replicate blood O_2 transport characteristics by designing HBOCs with similar Hb concentrations to normal blood [12]. However, most of these products were not further developed, as studies showed that they were more harmful than beneficial. From the first generation of HBOCs, we learned that acellular Hb depletes endothelial nitric oxide (NO) via an NO dioxygenase reaction that produces vasoconstriction and hypertension [13,14]. Other negative reactions partially responsible for vasoconstriction and hypertension exerted by first generation HBOCs were the extravasation of acellular Hb due to its small molecular size as well as metabolic regulation of blood flow in response to hyperoxygenation due to facilitated O_2 transport by the acellular Hb [15,16]. To overcome these issues, different strategies have been developed, including Hb polymerization and Hb surface decoration with polyethylene glycol (PEG) [17]. These materials

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* Corresponding author. Tel.: +1 858 534 5847.
E-mail addresses: dortizvelez@ucsd.edu (D. Ortiz), mmbarros@ucsd.edu (M. Barros), suyanmg@126.com (S. Yan), pcabrales@ucsd.edu (P. Cabrales).

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represent the second generation of HBOCs. They have demonstrated reduced vasoactivity, longer intravascular retention, minimal toxicity, and superior oxygenation [18,19]. Unfortunately, none of these products are currently approved for human use in the United States or Europe, mainly due to adverse events observed in clinical trials [20].

Microvascular function, in terms of blood flow and functional capillaries, is essential for the maintenance of tissue viability because the microcirculation is where O_2 is delivered and metabolic byproducts are washed out [21]. Hemoglobin-based O_2 carrier biophysical characteristics determine the mechanisms required to restore volume, microvascular blood flow, and O_2 delivery (DO_2). Hemopure (OPK Biotech, Cambridge, MA) is an HBOC developed originally by Biopure Corp (Cambridge, MA) that consists of polymerized bovine Hb. In 2004, the Food and Drug Administration suspended Hemopure's clinical trial involving surgical patients after safety concerns [22,23]. Regardless, Hemopure has been approved for clinical use in South Africa since 2006 and in Russia since 2012 [24]. Because of its long shelf life (up to 36 months) at room temperature and lack of compatibility concerns, Hemopure presents as solution to banked blood shortages in emergencies situations, natural disasters, and far forward sites where blood is not available. Therefore, more comprehensive research is necessary to define the appropriate administration, applicability, and strategies to mitigate adverse effects.

In this study, we investigate the effect of Hemopure Hb concentrations on systemic and microvascular parameters, including microhemodynamics and DO_2 during resuscitation from severe hemorrhagic shock compared to blood. Our results indicate that Hemopure favored the recovery of systemic parameters; however, microvascular function was compromised with higher concentrations of Hemopure compared to blood. Resuscitation with low doses of Hemopure was as effective as blood, suggesting that Hemopure or acellular HBOCs should be administered at a lower Hb dose relative to blood during resuscitation from hemorrhage to achieve a balance between enhanced DO_2 and tissue perfusion.

2. Methods

2.1. Materials

Hemopure was received as a donation from OPK Biotech.

2.2. Animal preparation

Male Syrian Golden hamsters weighting 55 to 70 g (Charles River Laboratories, Boston, MA) were fitted with a window chamber model. Animal handling and care followed the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The local animal care committee further approved the experimental protocol. The window chamber preparation presents a microvascular network of intact tissue that can be studied without anesthesia [25]. The tissues to be studied are composed of skeletal muscle and subcutaneous connective tissue and have been thoroughly described in the literature. Briefly, the animals were prepared for chamber implantation under anesthesia. The window chamber consists of 2 titanium frames with a 15-mm-diameter circular observation window. Sutures were used to lift the dorsal skin away from the animal, and 1 frame of the titanium chamber was positioned in contact with the animal skin. One side of the skinfold was removed following the outline of the window until only a thin monolayer of retractor muscle and subcutaneous skin of the opposing side remained. Then, a cover glass was placed on the exposed tissue and held in place by the other frame of the chamber under a drop of saline. The animals were allowed 2 days for recovery; then, each animal's chamber was assessed under the microscope for any signs of edema, bleeding, or unusual neovascularization. Barring these complications, the animal was anesthetized again, and arterial (carotid) and venous (jugular) catheters were implanted. The catheters were tunneled under the skin and exteriorized at the dorsal side of the neck where they were attached to the chamber frame for easy access. The animals were then allowed another day for recovery before the hemorrhagic shock experiments.

2.3. Inclusion criteria

Animals were considered suitable for experiments if (1) systemic parameters were within the reference range, namely, heart rate (HR) greater than 340 beats per minute, mean arterial pressure (MAP) greater than 80 mm Hg, systemic hematocrit (Hct) greater than 45%, and Pa_{O_2} greater than 50 mm Hg, and (2) microscopic examination of the tissue in the chamber observed under magnification ×20 did not reveal signs of edema, bleeding, or unusual neovascularization. Hamsters, fossorial animals that have adapted to an underground environment, have lower Pa_{O_2} than other rodents. However, microvascular P_{O_2} distribution in the chamber window model is similar to other rodents [26].

2.4. Hemorrhage and resuscitation protocol

Acute hemorrhage was produced by withdrawal of 50% of the blood volume (BV) via the carotid artery catheter within 10 minutes. The animal's BV was estimated as 7% of the animal's body weight. One hour after the hemorrhage, the animals received a single-bolus infusion of either an estimated 25% BV of Hemopure at different Hb concentrations or the shed blood. Hemopure Hb concentration was adjusted by dilution with lactated Ringer's solution. The parameters being analyzed were measured before hemorrhage induction (baseline), after hemorrhage (shock), and up to 90 minutes post volume replacement (resuscitation). A schematic timeline of the protocol is shown in Fig. 1B.

2.5. Resuscitation fluids and experimental groups

A total of 24 animals were included in the study. They were randomly assigned to each experimental group depending on the fluid used for resuscitation. The fluids used were (i) blood, shed blood from the same animal (group labeled Blood, $n = 6$) and (ii) Hemopure at 3 different Hb concentrations—4 g/dL (group labeled HBOC4, $n = 6$), 8 g/dL (group labeled HBOC8, $n = 6$), and 12 g/dL (group labeled HBOC12, $n = 6$).

2.6. Systemic hemodynamic and blood gas parameter

Mean arterial pressure and HR were continuously recorded (MP150; Biopac Systems, Santa Barbara, CA). Arterial blood collected in a heparinized capillary tube was immediately analyzed for Pa_{O_2}, Pa_{CO_2}, base excess (BE), and pH (Blood Chemistry Analyzer 248; Bayer, Norwood, MA). The Hct was measured from centrifuged arterial blood taken in heparinized capillary tubes. The Hb concentrations were measured spectrophotometrically using the B-Hemoglobin (Hemocue, Stockholm, Sweden). Hemocue's cuvettes hemolyze the RBC, coverts the Hb to azide-metahemoglobin, and corrects for cell debris to measure Hb concentration [27]. Total Hb was measured directly from a drop of arterial blood, and plasma Hb was measured using the plasma collected after Hct measurements. Hemocue has been validated for plasma Hb concentrations between 0.2 and 5 g/dL with unencapsulated HBOCs [28].

2.7. Microvascular experimental setup

The awake animals were placed in a restraining tube with a longitudinal slit from which the window chamber protruded and then