

Hb-based oxygen carriers: are we there yet?

Thomas J. Reid

In a recent issue of **TRANSFUSION**, Lanzkron and coworkers¹ describe a patient with sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, and acute chest syndrome who, on religious conviction, refused RBC transfusion. Improved oxygenation is the major goal of the treatment for acute chest syndrome,^{2,3} and this is accomplished with supplemental oxygen (O₂), bronchodilator therapy, and/or RBC transfusions.^{2,3} Multiple factors contributed to tissue hypoxia in this patient: diminished O₂ exchange (pulmonary embolism, pulmonary vasoocclusion from irreversibly sickled cells with sickling further exacerbated by hypoxia, and hypoxia-induced pulmonary vasoconstriction), O₂ transport (anemia), and O₂ delivery (methemoglobin subunits generated during oxidative stress in glucose-6-phosphate dehydrogenase deficiency led to a left-shifted, i.e., higher-affinity, O₂ dissociation curve). In such a patient, transfusion therapy would be expected to improve oxygenation by reducing the relative content of sickled cells through hemodilution (minimizing the procoagulant properties of irreversibly sickled cells⁴ and vasoocclusion) as well as enhancing O₂ delivery and exchange. As RBC transfusions were unacceptable to this patient, our clinical experience would suggest that the prospect of failing to improve oxygen delivery would have placed her at very high risk certainly for continued and worsening morbidity and possibly for mortality. This patient's reduced heart rate after transfusion of a solution of chemi-

cally modified Hb derived from human blood (PolyHeme, Northfield Laboratories Inc., Evanston, IL) is interpreted to reflect improved tissue oxygenation as a consequence of this intervention and her subsequent clinical course suggests a benefit from PolyHeme. Her recovery may have further been influenced through Hb-based oxygen carrier (HBOC) stimulation of erythropoiesis, an effect concluded from studies with other HBOCs.⁵ PolyHeme was well tolerated in this patient, a result supported in a report of asymptomatic sickle cell patients.⁶

The HBOCs are a class within the larger family of oxygen therapeutics that includes perfluorocarbon emulsions and liposome-encapsulated Hb. The primary function of all these agents is to provide oxygen-carrying capacity, whether serving as RBC substitutes or primary resuscitation solutions. The purpose of this review is to provide a brief history of the field, examine the current status of HBOCs under development, propose a clinical framework to study and use these products, and offer suggestions for the future.

HISTORY AND CURRENT STATUS

The initial development of RBC substitutes was precipitated by the needs of the military, for an improved resuscitation fluid and for a blood substitute, and universal concerns about blood safety. Although blood safety has improved significantly,^{7,8} the military need for a RBC substitute remains and additional incentives for continued development of HBOCs exist: periodic and anticipated blood shortages, situations where transfusions are ineffective (severe hemolytic anemias) or refused, toxicities experienced by some recipients of blood transfusions⁹⁻¹¹ (e.g., TRALI), and possible contributions of WBCs to multiple organ failure.¹² The optimal RBC substitute would be pathogen-free and universal (not require cross-matching and would therefore be free of potential clerical error). It would also have a long shelf life (up to years) over a wide range of ambient temperatures (e.g., -20 to +50°C), exhibit a long intravascular t_{1/2} (from hours to days), have an acceptable toxicity profile, and provide efficacy (however defined) no worse than that of RBCs.

Early attempts toward a RBC substitute using Hb in human recipients met with failure due to abdominal pain, short intravascular t_{1/2}, and nephrotoxicity.¹³ These difficulties have been attributed in large part to the affin-

ABBREVIATIONS: HBOC(s) = Hb-based oxygen carrier(s); RBCU(s) = RBC units; t_{1/2} = half-life.

From the Department of Blood Research, Walter Reed Army Institute of Research, Silver Spring, Maryland.

Address reprint requests to: Thomas J. Reid, MD, PhD, FACP, Department of Blood Research, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500; e-mail: thomas.reid@na.amedd.army.mil.

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ity of Hb for nitric oxide,^{14,15} dissociation of the $\alpha_2\beta_2$ tetramer to $\alpha\beta$ dimers that can be filtered through the renal glomeruli, and contamination with RBC stroma.^{16,17} To overcome the problems of these predecessors, several types of HBOCs have been in development over the past 20 years. All HBOCs manufactured today are free of contamination from RBC stromal elements. The first to reach advanced Phase III clinical trials, diaspirin cross-linked human Hb (HemAssist, Baxter Healthcare Corp., Boulder, CO [an identical product manufactured by the US Army was referred to as $\alpha\alpha$ -cross-linked Hb]), was made of Hb tetramers cross-linked between the alpha subunits with bis-(3,5-dibromosalicyl)fumarate to prevent dissociation into dimers and maintain a low oxygen affinity. Continued development of HemAssist was terminated when patients in the treatment arms in studies of stroke¹⁸ and traumatic hemorrhage¹⁹ showed an increased percentage of serious adverse events and mortality when compared to the control groups. HemAssist is vasoconstrictive and the cause of vasoconstriction is thought by many to be the binding of endothelial-derived nitric oxide to tetrameric Hb, which unlike the polymerized molecules, gains access to the subendothelium.²⁰ In some clinical situations, this vasoactivity may be undesirable because small amounts of the product may elevate the blood pressure without normalizing cardiac output or restoring intravascular volume. Other HBOCs may also demonstrate vasoactive properties, related in part to the amount of residual tetramer in the final product. Additional mechanisms potentially contribute to vasoactivity, such as rates of molecular diffusion and oxygen transfer²¹ and viscosity.²²

Hemolink (Hemosol, Inc., Mississauga, Ontario, Canada) is an *o*-raffinose polymerized human hemoglobin (a "raffimer") with residual unpolymerized tetramers. Hemolink has completed Phase III trials in cardiothoracic surgery in Canada and has ongoing Phase II studies in this same population in the US. The Canadian studies²³ demonstrate the effectiveness of Hemolink in reducing RBC unit (RBCU) requirements in coronary artery bypass graft patients. A pyridoxylated human Hb tetramer with its molecular surface altered by polyoxyethylene (PHP, Curacyte Inc., Durham, NC) exploits the vasoactive property to improve blood pressure in hypotensive septic patients.²⁴ After successful Phase II studies that showed efficacy, Phase III studies are under way. Because of its conjugation with the polymer polyoxyethylene, PHP also has the property of prolonged intravascular survival. Considering that the tetramers may be primarily responsible for the vasoactivity of Hb preparations, different approaches^{25,26} have been taken to reduce the vasoconstrictive properties to include polymerization, chemical modification of the surface, and genetic engineering of the heme pocket.

Two products are glutaraldehyde cross-linked poly-

mers ("glutamers") of bovine or human Hb (Hemopure, HBOC-201, Biopure, Cambridge, MA; and PolyHeme, Northfield Laboratories, Inc., respectively), in which two or more tetramers are covalently linked. Although these processes are designed to optimize cross-linking, some tetramer remains, and both manufacturers subsequently remove residual tetramer to varying degrees. Hemopure has been reported to significantly reduce the need for RBCU transfusion in patients undergoing cardiac²⁷ and abdominal aortic surgeries.²⁸ Hemopure was well tolerated as a resuscitation fluid given intraoperatively.²⁹ To replace 2,3-DPG, a pyridoxal molecule is incorporated into each tetramer of PolyHeme. In a Phase II study, this is the only product to date successfully given (up to 20 units) over a short period to trauma patients, some with RBC Hb concentrations below 30 g per L.^{30,31} PolyHeme has been shown to reduce the need for RBCU transfusions in trauma patients.³² A glutaraldehyde cross-linked bovine Hb product with covalently attached catalase and superoxide dismutase (McGill University, Montreal, Quebec, Canada) has been constructed in an attempt to prophylactically reduce ischemia-reperfusion injury³³ by removing reactive oxygen species (superoxide anion, hydrogen peroxide) and is in preclinical evaluation.

To eliminate the oxidative potential of Hb, Hb has been modified through intramolecular (with *o*-adenosine-5'-triphosphate) and intermolecular (with *o*-adenosine) cross-links as well as conjugation with reduced glutathione in the synthesis of a bovine-derived HBOC (HemoTech, HemoBioTech, Inc., Amarillo, TX). A series of nine patients with symptomatic sickle cell disease have been treated with HemoTech.³⁴ A polynitroxylated human Hb (HemoZyme, SynZyme Technologies, LLC, Irvine, CA) has also been designed to eliminate the oxidative potential of Hb and is in preclinical evaluation.^{35,36} Another product with a long $t_{1/2}$ (Hemospan, Sangart, Inc., San Diego, CA), conjugated with PEG,³⁷ has also been developed to control the vasoactive properties of tetrameric human Hb. This product will soon begin a Phase II clinical trial in the setting of elective surgery. Another product in preclinical studies^{38,39} (OxyVita, IPBL Pharmaceuticals, Inc., Goshen, NY) is formed by the polymerization of tetramers through intermolecular amide bonds,⁴⁰ after first cross-linking beta subunits with bis(3,5-dibromosalicyl)sebacate.⁴¹ Baxter's recent approach is the design of a genetically engineered Hb with diminished capacity to bind nitric oxide, yet with retained normal oxygen affinity.^{42,43}

In addition to vasoactivity, the unique redox chemistry,⁴⁴⁻⁴⁶ oncotic properties,⁴⁷ and intravascular $t_{1/2}$ of each of the Hb products influence the efficacy for specific clinical indications. The surface-conjugated HBOCs (Hemospan, PHP), for example, exhibit higher oncotic pressures and viscosities⁴⁸ as well as longer intravascular $t_{1/2}$ (48 vs. 24 hr) when compared to other preparations. Dis-

tinct from the physical and chemical properties of the HBOCs, it is important to recognize that a patient's underlying illness and clinical condition may also influence the physiologic responses to any of these agents. A summary of HBOCs under development is provided in Table 1.

No product has yet been approved in the US for any indication. Hemopure has been approved in South Africa for use as an alternate to RBC transfusion (for the purpose of avoiding RBCU transfusion) in general surgical patients—information on clinical use after regulatory approval is not yet available.

CLINICAL APPLICATIONS

The US FDA has clearly stipulated that demonstrations of safety and efficacy are absolute requirements for licensure of an HBOC.^{49,50} Over the past 10 years, clinical and preclinical investigations have enhanced our knowledge of the safety issues. As pointed out previously,⁵¹ efficacy is ill-defined because there is no comparative standard—the efficacy of RBCs has never been tested in human clinical trials. Moreover, there is a lack of consensus and indeed understanding of when RBCUs should be transfused, the so-called “transfusion trigger.”^{52,53} What may be an acceptable Hb level in otherwise healthy subjects^{54,55} could be problematic for severely ill patients, particularly those with comorbid medical problems.¹

The endpoints of efficacy are the major issues in the design of HBOC clinical trials and depend on the indicated use of an HBOC and expected clinical outcome. These endpoints may include mortality, transfusion avoidance (surgical and nonsurgical), or organ perfusion. The only endpoint the FDA has publicly suggested is that of transfusion avoidance and, consequently, this is the endpoint to which most manufactures have gravitated.⁵¹ With results trickling in from clinical trials, a better understanding of the products, and the absence of clearly established regulatory guidelines, it may be warranted at this time to suggest a broadly based framework in which to view HBOCs and their clinical indications, trial design, and possible endpoints demonstrating efficacy. Four broad categories of potential indications for HBOCs are proposed (Table 2): type I, as an alternative to RBCU transfusion; type II, as a primary resuscitative fluid; type III, when RBCUs are unavailable, contraindicated, or refused or in situations where the population of a particular clinical group is small; and type IV, for miscellaneous uses (radiation sensitization, prevention of TRALI, prevention of ischemia-reperfusion injury, and others). For each of these potential indications, different types of clinical trials with distinct endpoints would be appropriate.

In randomized studies comparing RBCUs and HBOCs, the latter show promise as an alternate to RBCU transfusions (type I indication) by significantly reducing

TABLE 1. HBOCs presently under development

Institution	Product name	Hb source	Chemistry	Clinical status in the US
Baxter Healthcare Corp.	rHb2.0*	Recombinant	Subunits engineered for reduced nitric oxide binding and maintenance of oxygen affinity	Preclinical
Biopure†	Hemopure‡§	Bovine	Glutaraldehyde-polymerized	Phase III, orthopedic and general surgery
Curacyte, Inc. (Apex Biosciences)	PHP	Human	Pyridoxylated tetramers conjugated with polyoxyethylene	Phase III, sepsis
HemoBioTech, Inc.	HemoTech	Bovine	Covalent attachment of reduced glutathione, adenosine, and adenosine-5'-triphosphate molecules	Preclinical
Hemosol, Inc.	Hemolink	Human	α-Raffinose-polymerized	Phase II, cardiothoracic surgery
IPBL Pharmaceuticals, Inc.	OxyVita	Human or bovine	Sebacoyl cross-linked tetramers and zero-length polymerized	Preclinical
Northfield Laboratories, Inc.†	PolyHeme	Human	Pyridoxylated tetramers and glutaraldehyde-polymerized	Phase II, completed trauma trials
Sangart, Inc.	Hemospan	Human	Tetramers conjugated with PEG	Phase II, elective surgery
SynZyme Technologies, LLC	HemoZyme	Human	Polynitroxylated tetramers	Preclinical
McGill University	PolyHb-SOD-CAT	Bovine	Tetramers copolymerized with catalase and SOD	Preclinical

* Their first-generation product, HemAssist, is no longer in clinical trials.

† Have submitted Biological License Application to the US FDA.

‡ Approved in the Republic of South Africa for use in general surgery.

§ A related product, Oxypure, has been approved in the US for veterinary use.

|| PolyHb-SOD-CAT = glutaraldehyde cross-linked bovine Hb product with covalently attached catalase and superoxide dismutase.

TABLE 2. Clinical indications for HBOCs

Indication type	Potential clinical indications	Appropriate trial design	Possible endpoints
I	Alternative to RBC transfusion—true RBC substitute (surgical and nonsurgical [e.g., chronic anemia] indications; trauma [prehospital vs. hospital use])	Randomized Phase III clinical studies comparing HBOC to RBCU	1) Reduction in requirement for RBCU transfusion (transfusion avoidance)* 2) Noninferiority, equivalence, or superiority in comparison to RBCU (morbidity, mortality)
II	Primary resuscitation fluid (e.g., perioperative resuscitation; prehospital use in trauma)	Randomized Phase III clinical studies comparing HBOCs to standard resuscitative fluids as initial therapy (i.e., before RBCU transfusion)	1) Reduction in requirement for subsequent RBCU transfusion (transfusion avoidance)* 2) Noninferiority, equivalence, or superiority in comparison to standard resuscitative fluids (morbidity, mortality) 3) Improvement in measures associated with hemorrhagic shock 4) Improved organ function (perfusion) 5) Reduction in sequelae from large volume resuscitation
III	RBCUs are unavailable, contraindicated, or refused (e.g., religious considerations) or other situations with small affected populations	Phase II studies with best available historical controls (preferably matched)	1) Morbidity 2) Mortality
IV	Other uses (e.g., radiation sensitization in oncology; prevention of TRALI; prevention of ischemia-reperfusion injury, especially in stroke; treatment of sepsis)	Randomized Phase III clinical studies to demonstrate superiority over existing standard treatment regimens	1) Morbidity 2) Mortality 3) Other endpoints as dictated by the proposed use

* Only transfusion avoidance has been suggested as an endpoint by the FDA.

the number of RBCUs needed for transfusion in surgery^{23,27,32} and trauma.³² The potential for HBOCs in the trauma setting is well recognized,⁵⁶⁻⁵⁸ and several HBOC manufacturers are considering clinical trials for trauma patients. There are no reports of studies for type I indications in nonsurgical patients (e.g., those with symptomatic chronic anemia). No study to date uses mortality as an endpoint for this indication.

The use of an HBOC as a resuscitation fluid (type II indication) should be considered separately from its use as an alternate to RBCs (type I indication) because the HBOC would be introduced earlier in the treatment strategy (i.e., when RBCUs would not be routinely recommended). Trials of this type should compare an HBOC to a standard resuscitative fluid. Controversy remains as to whether crystalloid or colloid is the better choice as the standard resuscitative fluid and in which specific clinical situations each is preferable. The physiologic function of HBOCs is more similar to colloidal solutions, making this an attractive comparison group. The HemAssist^{18,19} trials were type II studies, comparing this product to crystalloid rather than a colloid. Other studies suggest that HBOCs may be well tolerated in limited^{29,59} but possibly not large-volume⁶⁰ resuscitation. There are no human trauma studies (either hospital or before-hospital) of this type presently enrolling patients in the US; however, some are under consideration. As in the HemAssist^{18,19}

trials, these studies may consider a mortality endpoint. Preclinical studies for Type II indications continue and suggest a single HBOC has the potential for both low and high volume resuscitation.⁶¹⁻⁶³

Because there generally will be small numbers of patients in each of the clinical situations of type III indications, it may prove difficult to accrue patients to Phase III trials. Case reports have clearly suggested a benefit in those patients in whom RBCU transfusions were refused as in the patient discussed earlier¹ and by other patients⁶⁴ or contraindicated because of cross-matching issues as in a patient with autoimmune hemolytic anemia.⁶⁵ Phase II studies with historical control groups (best available, but preferably matched) should be considered valid sources of clinical data for licensure. An example is a recently reported clinical trial³⁰ of massively hemorrhaging patients, most with severe trauma; some had received up to 20 units of an HBOC. The design of the trial was not to demonstrate equivalence or noninferiority (i.e., not worse than) to RBCU but rather to evaluate whether, after infusion with standard resuscitation fluids, an HBOC may be effective when RBCUs are unavailable. When compared to historical controls (unmatched patient group who had refused transfusion therapy),⁶⁶ this HBOC appears to improve survival in patients with a RBC Hb level of less than 30 g per L. It is possible that other HBOC products will behave similarly. Type III indications in-

clude a subset of type I where adequate numbers of patients cannot be expected for adequate accrual to Phase III studies and settings where RBCUs cannot be given and withholding an oxygen therapeutic is contrary to best clinical judgment.

Numerous type IV indications can be envisioned, some unique to HBOCs. Clinical reports in this group are gradually being published. A recent account demonstrated the reversal of myocardial ischemia with an HBOC⁶⁷—in this setting, the Hb molecule by nature of its smaller size has access to an underperfused region not accessible to the RBC because of vasoocclusion. This mechanism is similar to that of enhanced radiation sensitization for cancer therapy where a Hb molecule may deliver oxygen⁶⁸ through small-caliber vessels. Once the risk factors for TRALI^{9-11,69} have been more clearly identified, HBOCs could be used to prevent TRALI in those patients identified at high risk for developing this complication because HBOCs appear to lack the neutrophil-activating properties of stored RBCUs^{70,71} and washing RBCUs is time-consuming and may not guarantee complete removal of the offending agent(s). Clearly the design of HBOC clinical trials must focus on the desired indication and endpoints.

The potential military application for HBOCs is clear—an effective HBOC could be life-saving when RBCUs are not available in the remote setting. By their nature, military operational settings cannot be reproduced in nonmilitary clinical trials. In the absence of information from Phase III trials in trauma, data from Phase II trauma studies (type II indication) could be considered in licensure, provided that clinical efficacy and safety are demonstrated—further support could come from other Phase III trials (surgical and nonsurgical) evaluating the HBOC under review. Phase III trauma trials for both type I and II indications might be designed to address questions pertinent to military and nonmilitary situations.

The shelf life of those HBOCs tested ranges from 1 to 3 years: shelf life depends on the storage temperature and it is expected that most products will remain functional even after exposure to severe environments, although with reduced shelf life at very high temperatures; some products can withstand several freezing-thawing cycles. The product shelf life for HBOCs exceeds that which is presently possible with RBCUs. All HBOCs are in a liquid formulation from Hb concentrations up to 13 g per dL and unit volumes ranging from 250 to 500 mL; none are available in a lyophilized formulation. The cost of an HBOC unit is anticipated to be approximately two to three times that of a single RBCU. Because of the light absorption properties, all HBOCs may, to varying degrees, interfere with light-dependent laboratory tests and adjustments in assay procedures must be made.⁷²

THE FUTURE

As evidence for efficacy of HBOCs gradually accumulates and products near licensure, a concern may develop whether the manufacture of those HBOCs derived from human Hb would exacerbate the shortage of RBCs. Crude guesses argue that there will be no additional strain on the blood supply; however, a definitive conclusion awaits robust economic analysis.

Each HBOC will locate its clinical niche as the characteristics of the individually designed products are better understood. Although not yet universally demonstrated, it is anticipated that the HBOCs as a class will share some common features (e.g. erythropoietic stimulation). However, in general, the HBOCs are not expected to be clinically equivalent, and as clinicians we will have a menu of HBOCs and other members of the oxygen therapeutic family from which to tailor our therapy (i.e., to select an agent for a specific indication). For example, those HBOCs with long $t_{1/2}$ could benefit those patients with chronic anemias requiring treatment. Furthermore, the long shelf life of HBOCs, some over a wide range of ambient temperatures, makes these products ideal for large capacity storage depots (within the US, worldwide in embassies and consulates, etc.) in preparation for disaster relief, potential contamination of existing blood supplies through chemical and/or biologic terrorism, and military deployments. It is unlikely that any single HBOC will serve as the holy grail of blood substitutes, but rather each will be a chalice of reduced luster yet with important clinical roles.

The unfavorable outcomes for the treatment groups in the HemAssist trials^{19,73} has not precipitated a halt to the development of HBOCs. Rather, this experience has led all participants to redouble their efforts for a better understanding^{46,74} of the chemistry, physiology, and pharmacology of those products in clinical trials as well as those under development and design. Furthermore, the HemAssist trials have resulted in a reassessment of clinical trial design for those patients with expected high mortality rates.⁷⁵ Although regulatory approval for HBOCs has been elusive, the conservative yet delicate and graceful ballet between development and licensure has proven beneficial for our patients.

So, are we there yet? With the recent submissions of two applications for product licensure after more than 30 years of effort, we are close—very close.

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