

Review

Hemoglobin-based oxygen carriers

A Gerson Greenburg¹ and Hae Won Kim²

¹Professor of Surgery, Brown University School of Medicine, Providence, Rhode Island, USA

²Director of Surgical Research, The Miriam Hospital, Providence, Rhode Island, and Assistant Professor of Surgery, Brown University School of Medicine, Providence, Rhode Island, USA

Correspondence: A Gerson Greenburg, aggreenb@lifespan.org

Published online: 14 June 2004

Critical Care 2004, **8(Suppl 2)**:S61-S64 (DOI 10.1186/cc2455)

This article is online at <http://ccforum.com/content/8/S2/S61>

© 2004 BioMed Central Ltd

Abstract

Transfusable fluids that may be used as alternatives to red blood cell transfusion offer the promise of preserving tissue perfusion and minimizing hypoxic cellular damage, and this promise may soon be fulfilled. Clinical testing of hemoglobin-based oxygen carriers has faced and met challenges involving molecular design, safety, efficacy, and regulatory requirements. Three leading candidates have emerged: two human (PolyHeme[®] and HemoLink[™]) and one bovine-based hemoglobin solution (Hemopure[®]). Because a survival benefit has been difficult to demonstrate, avoidance of allogeneic transfusion has been adopted as the standard efficacy end-point for these agents. An update on clinical trial status is provided, and the potential utility of hemoglobin-based oxygen carriers in surgery combined with intraoperative autologous donation is discussed.

Keywords blood substitutes, red blood cell transfusion, surgery

Introduction

Any review of modern transfusion therapy would be incomplete without considerable discussion of hemoglobin-based oxygen carriers (HBOCs). This abbreviation is the preferred term for the class of transfusable fluids also known as 'oxygen therapeutics'. The biologic goal of transfusion therapy is to preserve tissue perfusion in order to minimize the damage – both physiologic and structural – that results from prolonged or repeated episodes of ischemia [1,2]. Oxygen-carrying solutions with crystalloid and colloid elements may be effective in many clinical situations. For example, in a simple analytical model designed to illustrate how HBOCs might be used in surgery, a red blood cell (RBC) substitute of the first generation, currently in clinical testing, is projected to replace up to 60% of the current use of allogeneic RBCs [2].

A brief note on development of hemoglobin-based oxygen carriers

Efforts to find a replacement for blood reported in the medical literature can be traced back further than a century [3]. Although earlier proposals for oxygen therapeutics were imaginative and interesting, few came to fruition. Failure to

achieve success rested with the lack of scientific knowledge about blood; furthermore, the technology needed to create a useful blood substitute solution was lacking. The crisis of infectious disease transmission in the 1980s [4] generated renewed interest, and engendered within the development process a sense of urgency, because use of blood replacement fluids allows the adverse consequences of transfusions to be avoided. The potential for transmission of HIV and a more scientific approach to transfusion medicine stimulated development of a new generation of potentially useful solutions that could substitute for blood. However, although many of the blood substitutes showed promise in the initial preclinical testing phase, few survived the rigors of regulatory oversight and safety considerations. In the mid- to late 1980s as many as 12 solutions were proposed for use as HBOCs in the clinical setting.

Once the challenges of molecular stability and ensuring appropriate oxygen affinity were addressed, some compounds entered the complex world of clinical testing [5]. Through a variety of molecular modifications, more stable hemoglobin tetramers or polymers were created that effec-

Table 1

Characteristics of three hemoglobin-based oxygen carriers being tested in advanced clinical trials

Details	Product name		
	PolyHeme®	Hemopure® (HBOC-201)	HemoLink™ (Hb-raffimer)
Company	Northfield Laboratories (Evanston, IL, USA)	Biopure Corporation (Cambridge, MA, USA)	Hemosol Inc. (Toronto, Canada)
Modification method	Pyridoxylation and glutaraldehyde polymerization (polymer)	Glutaraldehyde polymerization (polymer)	Crosslinking with o-raffinose (oligomer)
Hemoglobin source	Human hemoglobin	Bovine hemoglobin	Human hemoglobin
Hemoglobin concentration (g/dl)	10	13	10
Molecular weight	Not reported (< 64 kDa; 1.0%)	Average molecular weight: 250 kDa	<64 kDa: <5% 64–500 kDa: >90% >500 kDa: <3%
pH	Not reported	7.6–7.9	7.5
P ₅₀ (torr)	26–32	38	52
Viscosity (cP)	Not reported	1.3	1.14
Shelf life	>1 year	>1 year	>1 year

HBOC, hemoglobin-based oxygen carrier; P₅₀, partial pressure of oxygen at which hemoglobin is 50% saturated.

tively minimized renal clearance of the protein [6]. By the early to mid-1990s it became clear that pure solutions of hemoglobin were needed as starting materials, and technologies were being developed to synthesize such compounds [7,8]. Chemical modifications were applied to achieve the most desirable molecular effects (i.e. improved persistence and lowered oxygen affinity), and it became possible to manufacture reasonably large quantities of pharmaceutical grade HBOC solutions [7]. Efforts to create modified hemoglobin through recombinant technology were undertaken, with varying degrees of success [6]. Unfortunately, these latter efforts proved to be prohibitively expensive. Scaling the process up to produce the quantities required to meet anticipated demands were feared to exceed the costs that could be sustained in the eventual marketplace. Nevertheless, this recombinant technology remains an excellent approach for future research endeavors, and efforts at genetically determined site-specific modification and cross-linking are ongoing, although they are confined to laboratory exercises [6]. Given the prolonged period of time required to take a laboratory concept through the regulatory process, another decade may pass before any agents arising from recombinant technology are proven effective and safe to market for general use.

Leading candidate hemoglobin-based oxygen carriers for regulatory approval

Three forms of HBOCs are in advanced testing stages in the regulatory approval process [9]. One bovine hemoglobin-based solution under development relies on the naturally low affinity of bovine hemoglobin for oxygen as a major rationale for its use. In addition, there are two human hemoglobin-based solutions – one a polymer and the other an oligomeric

solution containing a polymeric compound and some cross-linked hemoglobin tetramer. Beyond their ability to carry and deliver oxygen to tissues, these solutions have different physicochemical and physiological properties as well as different biologic activities. Characteristics of these three candidate HBOCs are summarized in Table 1.

Through complex regulatory processes, these products have been negotiating toward licensure for clinical use, including phase III testing, whereas other solutions have not progressed this far [10]. Lessons from unsuccessful efforts are difficult to glean because data from an unsuccessful experiment are rarely published. Thus, the field as a whole is often deprived of the opportunity to benefit from experience and is prone to repeat the mistake, if indeed it is a preventable error. Some of this information may be revealed when investigators gather to compare notes or present research findings, but it is not generally formalized or broadly disseminated.

One topic of active debate over the past decade was the definition of 'efficacy' as it relates to HBOCs. Demonstrating survival benefit in most clinical models is very difficult, and correlating oxygen delivery and use parameters with survival outcome is equally difficult. Borrowing from other areas of transfusion medicine, avoidance of allogeneic transfusion has almost become a universal marker of efficacy for those solutions currently undergoing clinical trials [6].

Recent clinical trials and current status of hemoglobin-based oxygen carriers

Both bovine and human HBOCs have recently been tested in phase II/III clinical studies (Table 2). PolyHeme® (Northfield

Table 2**Recently published clinical studies of three leading hemoglobin-based oxygen carriers**

Details	Product name		
	PolyHeme®	Hemopure® (HBOC-201)	HemoLink™ (Hb-raffimer)
Company	Northfield Laboratories (Evanston, IL, USA)	Biopure Corporation (Cambridge, MA, USA)	Hemosol Inc. (Ontario, Canada)
Indicated use	Trauma/urgent surgery	Cardiac surgery	Coronary artery bypass graft
Clinical trial status	Phase III	Phase III	Phase II/III
Study design	Multicenter, randomized, double blind	Multicenter, randomized, double blind	Multicenter, randomized, single blind
End-point	Mortality	Transfusion avoidance	Transfusion avoidance
Major findings	PolyHeme increased survival	Hemopure reduced allogeneic red cell transfusion	HemoLink reduced allogeneic red cell transfusion
Current status	Phase III mortality study	Filed BLA	Clinical study suspended pending further analysis
Reference	Gould and coworkers [11]	Levy and coworkers [12]	Hill and coworkers [19]

BLA, Biologic License Application.

Laboratories, Evanston, IL, USA), a human hemoglobin-based polymeric HBOC, has been shown to be effective in reducing mortality of patients with severe acute anemia [11]. When compared with severely anemic historical control individuals who refused allogeneic RBC transfusion on religious grounds, the PolyHeme group had a lower mortality at comparable erythrocytic hemoglobin concentration. Hemopure® (Biopure Corporation, Cambridge, MA, USA), a bovine polymeric hemoglobin, and HemoLink™ (Hemosol, Inc., Mississauga, Ontario, Canada), an oligomeric human hemoglobin-based HBOC, have been shown to reduce allogeneic RBC transfusion in patients undergoing cardiac surgery [12].

Currently, PolyHeme is being tested in a pivotal phase III pre-hospital trauma study [13]. The study proposes to demonstrate the safety and efficacy of PolyHeme in improving survival when it is used to treat severely injured bleeding trauma patients at the scene of injury and during transit to the hospital. Of note, this study will be conducted under the informed consent waiver provision. Because of the nature and extent of injuries, patients eligible for the study will be unable to provide informed consent. Federal regulations allow clinical research without informed consent under certain emergency settings. It is anticipated that over 700 patients will be enrolled in the study from approximately 20 level I trauma centers across the country.

In 2001, Hemopure (hemoglobin glutamer-250 or HBOC-201) was approved in South Africa for treatment of adult surgical patients who are acutely anemic and for the purpose of eliminating, reducing, or delaying the need for allogeneic RBC transfusion in these patients [14]. In October 2002, Biopure filed a biologic license application to the US Food

and Drug Administration to market Hemopure in the USA for a similar indication in orthopedic surgical patients. In August 2003, the Food and Drug Administration requested additional information [15], including clarification of certain clinical and preclinical data, before ruling on whether to allow marketing of the product.

Some of the trials were discontinued early, such as the diaspirin cross-linked hemoglobin study, which demonstrated higher mortality in treated patients than in nontreated ones [10].

In early 2003, Hemosol voluntarily suspended a phase IIb cardiac surgery study when it discovered an imbalance in the incidence of certain adverse cardiac events, with higher numbers occurring in the HemoLink-treated group. Hemosol elected to terminate the study and is currently investigating the cause of the imbalance [16], which is thought to be related to the higher rate of diabetes in the HemoLink-treated group.

In addition, Sangart Corporation (San Diego, CA, USA) has reported positive results from a phase Ib/II clinical trial in Sweden of Hemospan™ (MP4), a HBOC based on human hemoglobin conjugated to maleimide polyethylene glycol. The trial enrolled patients undergoing orthopedic surgical procedures [17].

Perioperative use of hemoglobin-based oxygen carriers

Given the sensitivity to transfusion-related illness and the general fear by the public and physicians of blood transfusions, it is common for patients to ask for alternatives to banked blood. Almost all of the paradigms and algorithms for

avoiding transfusion tend to be resource intensive and expensive, and still do not eliminate all risk. Alternatives can be sought, and use of HBOCs as part of intraoperative autologous donation (IAD) makes a great deal of sense.

In the IAD model of autologous transfusion, blood lost during surgery is recovered and reinfused into the patient. Using hemodilution and an oxygen therapeutic to replace some of the oxygen-carrying capacity of the RBCs removed minimizes blood lost during procedures. In addition, the potential for impaired tissue perfusion is lessened. In a phase II clinical trial using the IAD concept in cardiac surgery, decreased exposure to allogeneic RBCs was noted in the population randomly assigned to the HBOC arm. Moreover, the effect appeared to be sustained over time, beyond the immediate operative period, with a real proportion of the population never having exposure to allogeneic blood or blood products [18]. In light of these findings, there is promise for even the first generation of HBOCs to have an impact on patient care and outcome.

Conclusion

While trials continue with the three major solutions, research efforts continue to search for alternatives and for newer formulations and concepts to apply. As our understanding of the physiology of oxygen delivery and use improves, so will the concepts that underlie the design of HBOCs. As our appreciation of the physiology of shock and resuscitation at many levels grows, it is to be expected that the desire to create specific HBOCs to meet the needs of specific situations will grow. Furthermore, as clinical experience and the knowledge base grows, it will become increasingly possible to develop more effective therapies. The field remains exciting and relevant. Even with a better appreciation of when to transfuse a patient, the options of what to use remain few. The addition of HBOCs to the armamentarium is welcome and will be embraced because they have allowed us to focus on the underlying issues of when to transfuse and what to use to accomplish our goal.

Competing interests

AGG is a member of the Hemosol Scientific Advisory Committee, and has a financial interest in Hemosol, Inc.

References

- Greenburg AG: **Indications for transfusion.** In *Scientific American Surgery*. Edited by Wilmore DW, Cheung LY, Harken AH, Holcroft JW, Meakins JL. New York, NY: Scientific American, Inc.; 1989:1-19.
- Greenburg AG: **A physiologic basis for red blood cell transfusion decisions.** *Am J Surg* 1995, **Suppl 6A**:44S-48S.
- Cohn SM: **Blood substitutes in surgery.** *Surgery* 2000, **127**: 599-602.
- Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ: **The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study.** *N Engl J Med* 1996, **334**:1685-1690.
- Lieberthal W, Fuhro R, Freedman JE, Toolan G, Loscalzo J, Valeri CR: **O-rafifinose cross-linking markedly reduces systemic and renal vasoconstrictor effects of unmodified human hemoglobin.** *J Pharmacol Exp Ther* 1999, **288**:1278-1287.

- Reid TJ: **Hb-based oxygen carriers: are we there yet?** *Transfusion* 2003, **43**:280-287.
- Lieberthal W, Fuhro R, Andry C, Valeri CR: **Effects of hemoglobin-based oxygen-carrying solutions in anesthetized rats with acute ischemic renal failure.** *J Lab Clin Med* 2000, **135**:73-81.
- Carmichael FJ, Ali AC, Campbell JA, Langlois SF, Biro GP, Willan AR, Pierce CH, Greenburg AG: **A phase I study of oxidized raffinose cross-linked human hemoglobin.** *Crit Care Med* 2000, **28**:2283-2292.
- Jahr JS, Nesargi SB, Lewis K, Johnson C: **Blood substitutes and oxygen therapeutics: an overview and current status.** *Am J Ther* 2002, **9**:437-443.
- Sloan EP, Koenigsberg M, Gens D, Cipolle M, Runge J, Mallory MN, Rodman G Jr: **Diaspirin cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock: a randomized controlled efficacy trial.** *JAMA* 1999, **282**:1857-1864.
- Gould SA, Moore EE, Hoyt DB, Ness PM, Norris EJ, Carson JL, Hides GA, Freeman IHG, DeWoskin R, Moss GS: **The life-sustaining capacity of human polymerized hemoglobin when red cells might be unavailable.** *J Am Coll Surg* 2002, **195**:445-452.
- Levy JH, Goodnough LT, Greilich PE, Parr GV, Stewart RW, Gratz I, Wahr J, Williams J, Comunale ME, Doblal D, Silvay G, Cohen M, Jahr JS, Vlahakes GJ: **Polymerized bovine hemoglobin solution as a replacement for allogeneic red blood cell transfusion after cardiac surgery: results of a randomized, double-blind trial.** *J Thoac Cardiovasc Surg* 2002, **12**:35-42.
- Northfield Laboratories Inc.: **Northfield reaches agreement with FDA on special protocol assessment for pivotal phase III trial.** [<http://www.northfieldlabs.com/releases/061203.htm>]
- Tshepo Pharmaceuticals (Pty) Ltd.: **Hemopure® (Hemoglobin Glutamer-250 [Bovine]) [Prescribing Information].** Pretoria, South Africa: Tshepo Pharmaceuticals (Pty) Ltd.; 2001.
- Biopure receives FDA response to hemopure(R) marketing application. [http://www.corporate-ir.net/ireye/ir_site.zhtml?ticker=BPUR&script=410&layout=0&item_id=437651]
- Hemosol Inc.: **Hemosol announces second quarter 2003 financial results.** [<http://hemosol.com/companynewsreleases.cfm?newsID=1919&companyID=24>]
- Sangart reports positive Ib/II clinical trial results for Hemospan™. [<http://www.sangart.com/press/view.cfm?ID=26>]
- Cheng DCH: **Safety and efficacy of o-rafifinose cross-linked human hemoglobin (Hemolink™) in cardiac surgery.** *Can J Anaesth* 2001, **Suppl**:S41-S48.
- Hill SE, Gottschalk LI, Grichnik K: **Safety and preliminary efficacy of hemoglobin raffimer for patients undergoing coronary artery bypass surgery.** *J Cardiothorac Vasc Anesth* 2002, **16**: 695-702.