Blood substitutes

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Purpose of review

Risk of disease transmission and limitations in the ability to transfuse in the prehospital or combat setting have stimulated research in the field of oxygen therapeutics. Several products have completed safety trials and are presently undergoing investigation for their efficacy. In the near future, the clinician will likely employ these products in the management of a variety of patient populations. Though similar in their oxygen carrying capacity, each agent possesses distinct physiologic effects. Understanding of the benefits and shortcomings of the various compounds is essential in order to optimally utilize them in various clinical settings. This review provides an overview of recent developments in the field of oxygen therapeutics and highlights results of clinical trials.

Recent findings

Modified hemoglobin solutions of human or bovine origin and perfluorochemical-based emulsions are in advanced stages of clinical testing. Bovine hemoglobin-based solutions have been associated with vascular reactivity, methemoglobin formation and development of antibodies. Larger safety trials are necessary before they can find widespread use. Polymerized human hemoglobin solutions have a favorable safety profile in early trials and have been effective as a resuscitation fluid in circumstances when red cells may be unavailable. Unfortunately, outdated human blood, the substrate for this product, is itself in short supply. Perfluorocarbons similarly reduce the need for allogeneic transfusion, but the need for high-inspired oxygen levels currently limits use. Recombinant, polymer-encapsulated and additional forms of chemically modified hemoglobins are being developed and are undergoing testing in animal models

Summary

Oxygen carriers offer a viable alternative to allogeneic transfusion. All oxygen therapeutic agents are not clinically equivalent. Optimal utilization requires a thorough understanding of the therapeutic potentials and adverse effects of the solution being considered for use.

Keywords

red cell substitutes, hemoglobin-based oxygen carriers, oxygen therapeutics, perfluorochemicals

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Abbreviations

DCLHb diasprin-crosslinked hemoglobin
HBOC hemoglobin based oxygen carrier

0952-7907

Introduction

The term 'blood substitutes' is a misnomer [1]. Current research focuses on solutions that possess the oxygen carrying capacity of blood but lack its numerous other functions. For this reason, these solutions are more aptly termed 'oxygen therapeutics' or 'oxygen carriers'. Concerns regarding transmission of infectious disease and the logistic problems with providing allogeneic blood transfusion in the combat setting have stimulated both civilian and military investigators in the development of an effective oxygen carrier. Unfortunately, early products were highly toxic. Over the last two decades, better understanding of the physiology of these solutions has led to the development of safer compounds. Several of these oxygen carriers have completed safety trials and are presently undergoing efficacy trials. It is only a matter of time before oxygen carriers become available for routine clinical use [2]. Clinicians must recognize that all oxygen carriers are not clinically equivalent, potential benefit must be balanced against their adverse effect profile and choice of the agent tailored to the particular situation. It is therefore imperative to have a clear understanding of the various oxygen carriers, their potential toxicities and current clinical applications.

This review provides a current overview of the field, with an emphasis on the most recent clinical trials involving the spectrum of oxygen carriers in advanced stages of clinical testing.

History of blood substitutes

The concept of blood substitutes is not new. Sir Christopher Wren suggested the possibility of replacing blood with ale over 400 years ago [3]. More scientific efforts at developing an effective blood substitute began over half a century ago when Amberson in 1949 administered a hemoglobin-saline solution as a last resort to a woman with severe postpartum hemorrhage. Though initially efficacious in restoring perfusion pressures, the patient subsequently developed irreversible renal failure and died. The renal toxicity observed was presumed to result from stromal contamination of the solution and subsequent attempts focused on the

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development of stroma free preparations of hemoglobin. Despite purification, the renal toxicity remained. Recognition of the role of dimeric hemoglobin in the pathophysiology of the observed renal failure led to interest in the development of chemically modified hemoglobin that would retain their tetrameric form and thereby have longer intravascular retention and be free from renal toxicity. Growing concerns over transmission of disease by allogeneic blood transfusions and the need for a more practical, logistically useful oxygen carrier in combat situations provided the impetus for continued research in this field. Much of this effort has been directed towards the development of chemically modified hemoglobin solutions. As problems with renal toxicity have been overcome, the vasconstrictive effects of nitric oxide scavenging are only now being fully recognized. Despite these setbacks, several oxygen carriers are in phase III clinical trials and their incorporation into clinical practice both as a resuscitation fluid and as an alternative to allogeneic blood transfusion appears imminent.

Current rationale for the development of blood substitutes

There are several potential advantages of an oxygen therapeutic agent when compared with allogeneic blood transfusion: (1) elimination of the potential transmission of infectious agents, notably HIV and hepatitis C virus; (2) elimination of the need for a time-consuming cross-match as well as, possibly, transfusion reactions, leading to utilization of the same solution in patients of all blood types; (3) long shelf life; (4) lack of the deleterious effects of time on banked blood, the so-called 'storage-lesion' [4]. (5) they do not require specialized storage conditions; (6) ability to pharmacologically alter oxygen transport characteristics; (7) improved microcirculatory flow. A long shelf life and lack of need for specialized storage conditions would make widespread availability a reality especially in rural areas without sophisticated blood banking centers as well as in forward surgical units of the military. The storage lesion is cause by a reduction in 2,3-diphosphoglycerate in stored blood over time, resulting in an increased affinity for hemoglobin with compromised oxygen delivery to the tissues. Red cell pliability also decreases over time, reducing microcirculatory flow and life of the transfused red cell. Additionally, it is recognized that red cell transfusion may lead to altered immune function likely as a consequence of inadvertent transmission of leukocytes. This effect has been implicated in the increased risk of developing infections [5] as well as recurrence of cancers. Finally the ability to pharmacologically alter the oxygen loading and unloading characteristics as well as improved rheologic properties are other potential desirable advantages of these compounds.

Potential application of blood substitutes

Oxygen carriers are potentially useful in the setting of resuscitation of trauma, hemodilution before or during surgery to reduce or eliminate the need for allogeneic red cell transfusion, as an adjunct to the management of severe anemia and in specialized functions such as the pump prime for cardiopulmonary bypass. They may serve as a bridge to transfusion when blood may be unavailable, as in the battlefield, or when its availability may be delayed as with difficult cross-matches and rare blood types. Their potential to alleviate the relative shortage in the blood supply as need outstrips supply especially in times of acute crisis is particularly attractive in view of current world events [6].

Classification of blood substitutes

There are two major categories of blood substitutes under evaluation currently: hemoglobin-based oxygen carriers and perfluorocarbon-based products (Table 1). The HBOCs are further classified into chemically modified, recombinant, and encapsulated forms of hemoglobins. Of these types of hemoglobin, only chemically modified forms are in advanced stages of clinical trials and will be discussed further.

Perfluorocarbons are synthetic fluorinated hydrocarbon molecules that increase the amount of oxygen dissolved in the fluid phase in a manner proportional to the partial pressure of oxygen.

Physiologic effects of significance

The ability of these oxygen carriers to transport oxygen accounts for their therapeutic utility. They also possess several other physiologic effects that contribute to their limitations and adverse effect profile. While these effects vary with the specific product under study, some generalizations can be made.

Most important is their pressor effect, more frequently observed with modified human hemoglobins. This effect is believed to be multifactorial and results from a combination of nitric oxide scavenging, release of endothelin and upregulation of the peripheral α-adrenergic receptors. Certain forms of oxygen carriers appear to be free of these vasoconstrictive effects, probably as a result of extensive polymerization of the compounds with less than 1% unpolymerized tetrameric hemoglobin [7••].

Effect on the nitric oxide mediated modulation of smooth muscle contraction produces abdominal pain and esophageal dysmotility. Alterations in regional blood flow are believed to result in the elevation of amylase and lipase that is observed, without clinical evidence of pancreatitis.

Table 1. Classification of oxygen carriers

Carrier	Current Status
Hemoglobin-based oxygen carriers	
Human hemoglobin based	
PolyHeme (Northfield Laboratories Inc.) Chicago, IL, USA	Phase III Ongoing general surgery and vascular surgery trials. Initiating trauma trials with the US Army.
Hemolink (Hemosol Inc.) Toronto, Ontario, Canada	Phase III Completed trials in CABG patients in the UK and Canada.
Hemospan (Sangart Inc.) San Diego, CA, USA	Phase II
Hemozyme (SynZyme Technologies LLC) Irvine, CA, USA	Preclinical
HemAssist (Baxter) Chicago, IL, USA	Withdrawn
Bovine hemoglobin based	
Hemopure (Biopure Inc.) Biopure Corporation, Cambridge, MA, USA	Phase III Completed orthopedic trials. BLA submitted to the FDA. Trauma trials initiating with US Navy.
HemoTech (HemoBioTech Inc.) Lubbock, TX, USA	Preclinical
PolyHb-SOD-CAT (McGill University)Montreal, Quebec, Canada	Preclinical
Recombinant hemoglobin based	
Optro (Baxter) Chicago, IL, USA	Withdrawn
rHb2.0 (Baxter) Chicago, IL, USA	Withdrawn
Encapsulated hemoglobin	Preclinical
Perfluorocarbon-based oxygen carriers	
Perflubron emulsion (Alliance Pharmaceuticals) San Diego, CA, USA	Terminated phase III trials

CABG, coronary artery bypass graft. BLA, biologics license application; FDA, Food and Drug Administration.

Oxidation of the heme moiety of hemoglobin during its degradation results in the formation of methemoglobin. Its accumulation is a limiting factor in the dose and frequency of administration.

The issue of interference with clinical laboratory testing due to calorimetric changes of the dissolved plasma hemoglobin has not proved to be a clinically significant problem.

Current clinical trials

Several of the oxygen carriers have completed safety trials and are at the present time undergoing evaluation for their efficacy. As these products are not clinically equivalent, results of the most recent trials of each individual solution in advanced phases of testing will be discussed separately.

Diasprin-crosslinked hemoglobin

Schubert *et al.* [8] in a prospective double-blind multicenter study included patients undergoing orthopedic, abdominal or vascular surgery with high anticipated blood loss to receive either diasprin-crosslinked hemoglobin (DCLHb) or allogeneic blood if transfusion criteria were met within 36 h of surgery. The primary endpoint was percentage of DCLHb patients spared allogeneic transfusion and the median transfusion requirements in the study versus the control group. At 18 months, with 181 patients enrolled the study was prematurely terminated for two reasons: the development of serious adverse events in two patients and unfavorable results from the interim analysis of a parallel trauma trial [9]. At the time of termination of the study, 23% of patients in the DCLHb group were completely spared allogeneic transfusion in the first 7 days following surgery. This effect was most pronounced early and decreased over time, likely reflecting the limited half-life of the DCLHb. The median difference in volume of allogeneic blood transfused was 250 ml less in the DCLHb group, a difference that though statistically significant was of unknown clinical significance.

Kerner *et al.* [10] similarly reported recently on an abbreviated multicenter European trial that evaluated the effect of 'on-scene' administration of DCLHb. The augmented tissue oxygen delivery, it was postulated, would prevent the development of early multiple organ failure, reduce the use of blood products and 28-day mortality. While the study did demonstrate a reduction in blood product usage in the first 24 h, this difference disappeared by 7 days. Further, there was no improvement in early organ failures, no decrease in early deaths or mortality at 28 days. Failure to demonstrate efficacy and concerns of safety led to the premature termination of this trial.

DCLHb has been withdrawn from all clinical trials. A second-generation blood substitute under development was undergoing animal studies. Recently, Baxter terminated all research and development in this arena.

Polymerized human hemoglobin

Gould *et al.* [7••] administered up to 20 units of PolyHeme (Northfield Laboratories, Chicago, IL, USA) to patients undergoing resuscitation for either trauma or

urgent surgery. Thirty-day mortality was compared with a historical group consisting of patients undergoing surgery with hemoglobin levels less than 8 g/dl that refused red cell transfusions on religious grounds. Not only did the administration of PolyHeme maintain the total hemoglobin (red cell hemoglobin + PolyHeme hemoglobin) above the recommended 7–10 g/dl, 30-day mortality was significantly better (25.0% PolyHeme versus 64.5% in historical controls) in patients with life-threatening red blood cell hemoglobin levels. There were no significant adverse events related to the infusions. The utility of PolyHeme as an adjunct to acute normovolemic hemodilution during aortic surgery with large volume of blood loss has also been recently described [11]. A 78-year-old man underwent repair of a juxta-renal abdominal aneurysm and bilateral iliac artery aneurysms. Acute normovolemic hemodilution was performed using PolyHeme as the replacement fluid to maintain the total hemoglobin level. It was further utilized to replace ongoing surgical blood loss for the majority of the case, following which the harvested blood was transfused. No allogeneic blood transfusion was needed intraoperatively or postoperatively despite a 4000 ml blood loss. No serious adverse events were noted as a result of this approach. It was used successfully in the management of acute chest syndrome in a patient with sickle cell crisis who refused red cell transfusion on religious grounds [12•]. In addition to being efficacious, PolyHeme is reportedly free of the vascoactive effects that plague other forms of hemoglobin-based oxygen carriers.

PolyHeme is currently undergoing phase III trials in general surgical patients, and trauma trials are being initiated.

Bovine hemoglobin glutamer-250

Bovine hemoglobin glutamer-250 [Hemopure; HBOC-201 (Biopure Corporation, Cambridge, MA USA)], has been used successfully in the reduction of allogeneic blood transfusion in patients undergoing elective cardiac, aortic and hepatic surgery [13•]. Sprung *et al.* [14•] recently reported the results of a multicenter, randomized single-blind trial to evaluate the safety of a single dose of HBOC-201 in patients undergoing a variety of major surgical procedures. Administration of escalating doses of HBOC-201 (0.6–2.5 g/kg) was generally well tolerated with no serious adverse events. Patients in the study group had higher systemic blood pressures (approximately 14 mmHg), greater incidence of dermatologic adverse effects, significantly higher mean methemoglobin levels on day 3 and a 57% incidence of immunoglobulin G antibodies to HBOC-201 at 2 weeks. The improved hemodynamic properties observed were not associated with a reduction in transfusion requirements. The basis for the hemodynamic changes observed is unclear and may represent either the effect of nitric oxide binding or a consequence of volume expansion. Methemoglobin levels rose in a delayed manner suggesting ongoing oxidation of the plasma hemoglobin as the likely mechanism. While the levels were not clinically significant even at the higher doses of HBOC-201 (7.7% at a 2.5g/kg dose), methemoglobinemia may become a problem in future efficacy trials that require even larger doses. The occurrence of immunoglobulin G antibodies in over half of the patients also raises concern should the need for repeated administration arise.

Levy *et al.* [13•] studied the efficacy of HBOC-201 in avoiding the need for postoperative (first 72 h) red cell transfusion in patients undergoing uncomplicated cardiac surgery. Thirty-four percent of patients given HBOC-201 did not require subsequent red cell transfusion during the study period. The mean number of units transfused was also reduced by 0.47 red blood cell units per patient ($P = 0.05$).

This product has been approved for use in South Africa and results of their clinical experiences are eagerly awaited. The US Food and Drug Administration is responding to the recent biologics license application submission by Biopure. Phase III trials with this bovine product in trauma patients are being initiated.

Hemoglobin raffimer

Hill *et al.* [15] studied the tolerance of escalating doses of hemoglobin raffimer, avoidance of the need for red blood cell transfusion and the safety of this approach. Although the difference in rate of red cell transfusion was not statistically significant (44 versus 18%; $P = 0.093$), the median volume of red cells transfused was lower in the treatment group than in controls (550 ml versus 1175 ml; $P = 0.042$). Hypertension occurred more frequently in the treated group and was more common when higher doses of study medication were used, suggesting a dose response relationship. Study patients developed a transient elevation in liver enzymes and serum creatinine. These may represent subclinical toxicities that could become more fully manifest at higher doses. This warrants extreme caution in patient selection and monitoring in future trials. The significance of similar elevations in pancreatic enzymes without clinical evidence of pancreatitis needs to be elucidated. Antibody formation was observed in 21% of patients. While there was no clinical consequence in the short term, long-term effects remain undetermined. Given the small number of patients in the study, larger trials are necessary before the safety of this blood substitute can be confirmed. Phase III trials have been completed in

Canada and the United Kingdom and further work is expected.

Perflubron

Spahn *et al.* [16•] reported results of a phase III multicenter study evaluating the efficacy of perflubron emulsion to reduce allogeneic red cell transfusion in patients undergoing high-blood-loss non-cardiac surgery. In the study group, one dose of perflubron emulsion was administered at time of skin incision, and a second small dose administered if the hemoglobin levels fell to 6.5 ± 0.5 g/dl. A need for further transfusions was met with autologous or allogeneic blood. Overall, a significant reduction in transfusion requirements was noted in the first 24 h following surgery. This difference was no longer significant at 3 days. In the protocol-defined subgroup with blood loss of 20 ml/kg or higher, the reduction in transfusion remained significantly lower than controls through discharge. Hypertension and ileus were significantly more common in the perflubron treated group. Interestingly, no difference in occurrence of infectious complications was observed between the groups, suggesting that the theoretical concern of immune system dysfunction secondary to reticuloendothelial clearence of the perflubron emulsion particles is unfounded. Use of perflubron in the setting of autologous blood harvesting is therefore an attractive alternative to conventional allogeneic transfusion. While effective in the in-hospital setting, the need for a high-inspired oxygen tension may limit its utility in the prehospital or combat arena. A large phase III trial in cardiac bypass surgery patients was terminated after the unexpected finding of statistically significant lower incidence of neurologic complications in the control group compared with the experimental group receiving perflubron as part of perioperative normovolemic hemodilution.

Conclusion

Not all oxygen carriers are the same. None of the products has been used in large volumes or in a significant number of patients, limiting our understanding of their associated safety. Both the human and the bovine hemoglobin-based products appear effective at carrying oxygen in a manner similar to red blood cells.

Future research is needed to evaluate the safety of using larger volumes of oxygen carriers to completely avoid allogeneic transfusion. Combination of oxygen carriers with other products that carry out some of the other functions of blood, such as platelet substitutes [17] and coagulation factors [18], is another attractive option. Finally, efforts continue to develop further generations of more effective, less toxic hemoglobin-based oxygen carriers. The future of oxygen therapeutics is indeed promising.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Cohn SM. Oxygen therapeutics in trauma and surgery. J Trauma 2003; 54:S193–S198.

2 Reid TJ. Hb-based oxygen carriers: are we there yet? Transfusion 2003; 43:280–287.

3 Squires JE. Artificial blood. Science 2002; 295:1002–1005.

4 Berezina TL, Zaets SB, Morgan C, *et al.* Influence of storage on red blood cell rheologic properties. J Surg Res 2002; 102:6–12.

5 Offner PJ, Moore EE, Biffl WL, *et al.* Increased rate of infection associated with transfusion of old blood after severe injury. Arch Surg 2002; 137:711–717.

6 Ouellette G. The experience and actions of the Washington, DC, blood community on and after September 11. Presentation at the HHS Advisory Committee in blood safety and availability. What lessons can be learned from the events of Sept 11, 2001, that would strengthen the safety and availability of the US blood supply? Transcript of the 16th meeting. Vol 1. 31 January 2002; http://www.hhs.gov/bloodsafety/transcripts/20020131.html. [Accessed September 2003].

7 •• Gould SA, Moore EE, Hoyt DB, *et al.* The life-sustaining capacity of human polymerized hemoglobin when red cells might be unavailable. J Am Coll Surg 2002; 195:445–455.
Survival was compared in anemic patients refusing transfusion for religious reasons who received PolyHeme were compared with historical controls in this retrospective study. The 30-day mortality in patients with a RBC [Hb] $\leqslant 3$ g/dl who received the oxygen-carrier was 25% compared to 64.5% for controls.

8 Schubert A, Przybelski RJ, Eidt JF, *et al.* Diasprin-crosslinked hemoglobin reduces blood transfusion in noncardiac surgery: A multicenter, randomized, controlled, double-blinded trial. Anesth Analg 2002; 97:323–332.

9 Sloan EP, Koenigsberg M, Gens D, *et al.* Diasprin cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock: A randomized controlled efficacy trial. JAMA 1999; 282:1857–1864.

10 Kerner T, Ahlers O, Veit S, *et al.* DCL-Hb for trauma patients with severe hemorrhagic shock: the European ''On-Scene'' multicenter study. Intensive Care Med 2003; 29:378–385.

11 Norris EJ, Ness PM, Williams M. Use of a human polymerized hemoglobin solution as an adjunct to acute normovolemic hemodilution during complex abdominal aortic reconstruction. J Clin Anesth 2003; 15:220–223.

12 • Lanzkron S, Moliterno AR, Norris EJ, *et al.* Polymerized human Hb use in acute chest syndrome: a case report. Transfusion 2002; 42:1422–1424.
This discusses the utility of oxygen-carriers in acute chest syndrome in a patient with sickle cell disease. It introduces the potential to employ these solutions in other situations when an increase in oxygen carrying capacity may be desirable.

13 • Levy JH, Goodnough LT, Greilich PE, *et al.* Polymerized bovine hemoglobin solution as a replacement for allogeneic red blood cell transfusion after cardiac surgery: Results of a randomized, double-blind trial. J Thorac Cardiovasc 2002; 124:35–42.
A randomized, double-blind efficacy trial involving 98 patients demonstrated an elimination of the need for allogeneic red cell transfusion in 34% of cases.

14 • Sprung J, Kindscher JD, Wahr JA, *et al.* The use of bovine hemoglobin glutamer-250 (Hemopure) in surgical patients: Results of a multicenter, randomized, single-blinded trial. Anesth Analg 2002; 94:799–808.
This study demonstrated tolerance of HBOC-201 in patients undergoing surgery who received a single administration of escalating doses of the compound.

15 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.

16 • Spahn DR, Waschke KF, Standl T, *et al.* Use of perflubron emulsion to decrease allogeneic blood transfusion in high-blood-loss non-cardiac surgery. Anesthesiology 2002; 97:1338–1349.
This showed a significant decrease in allogeneic red cell transfusion in patients receiving perfulobron in high blood loss surgery.

17 Lee DH, Blajcham MH. Novel treatment modalities. New platelet preparations and substitutes. Br J Hematol 2001; 114:496–505.

18 Schraber MA, Holcomb JV, Hedner U, *et al.* The effect of recombinant factor VII on coagulopathic pigs with grade V liver injuries. J Trauma 2002; 53:431–439.