

Current Artificial Oxygen Carriers and Their Potential Role in the Management of Hemorrhage

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Learning Objectives: 1) To recite relevant definitions of blood substitutes, with regard to both hemoglobin oxygen carriers (HBOCs) and perfluorocarbons, as well as desirable characteristics of blood substitutes. 2) To relate structural modifications of the hemoglobin molecule to clinically important properties of HBOCs. 3) To compare HBOCs currently in human trials with respect to their performance in major surgical bleeding and hemorrhagic shock. 4) To prioritize potential complications and side effects of blood substitutes in clinical practice, with special emphasis in clinical situations where organs are already at risk. 5) To summarize the information available on the efficacy of blood substitutes to reduce or avoid allogeneic blood transfusions. 6) To list potential caveats and risks involved in the potential use of HBOCs for major blood loss.

Abstract
The field of viable artificial oxygen carriers, which includes both hemoglobin oxygen carriers (HBOCs) and perfluorocarbons, has narrowed considerably. The structure-activity relationships of HBOCs are reviewed, as are their side effects. A new HBOC (MalPEG-Hb) has been developed based on recent insights into microcirculatory oxygen unloading. Of the HBOCs left standing, a polymerized hemoglobin (PolyHeme) appears to hold some promise for clinical use in high blood-loss situations, based on clinical trials in acute trauma. Clinical investigative work relating to blood substitute performance in acute hemorrhage and for blood-sparing applications is discussed. Still, much work remains to be accomplished to document the safety of large-scale blood replacement with artificial oxygen carriers in humans.

Definitions

Technically, a blood substitute is a substance that can effectively replace all or most functions of human blood. However, oxygen-carrying modified hemoglobin solutions and perfluorocarbons have also been referred to as *blood substitutes*. Because these recently developed solutions can perform only selected functions of blood, they are more accurately referred to as *oxygen-carrying volume expanders*.

Hemoglobin-based oxygen carriers (HBOCs) are modified or packaged hemoglobin solutions that deliver oxygen to tissues. A hemoglobin therapeutic is a hemoglobin solution optimized through chemical modification to bring about certain additional pharmacologic and therapeutic effects. Hemoglobin therapeutics may possess a combination of therapeutically active properties such as oxygen-carrying capacity, favorable rheologic properties, or pressor action.

Need for Blood Substitutes

Although blood transfusions represent a life-saving measure for many medical and surgical patients, homologous blood transfusions are still not risk-free even in the United States. Oxygen-carrying volume expanders may be particularly helpful in situations in which blood is not available (e.g., remote areas, difficult cross-match, rare blood type) or in which blood is associated with adverse reactions, such as transfusion-associated lung injury. Furthermore, a national blood shortage is predicted with the aging of America, because older patients have a high demand for blood. The over-65 years age group represents about 12.5% of the population but receives 50% of all blood transfusions. Alternatives to blood transfusion would help cope with the predicted shortage. The risk of infection from blood has decreased dramatically, but potentially could be eliminated with blood substitutes. It is recognized, however, that prions and other still-undetected infectious agents could resist sterilization procedures employed in blood substitute production. Furthermore, blood transfusion can lead to higher mortality. Components of the postinjury hyperinflammatory response as measured by polymorphonuclear monocyte cytotoxicity,¹ and cytokines² were reduced in HBOC-resuscitated trauma patients compared with red blood cells. A less restrictive allogeneic blood transfusion strategy is associated with higher mortality in critical care patients less than 55 years old, compared with a more restrictive transfusion regimen.^{3,4}

Desirable “blood substitutes” have a long shelf-life, a long circulation half-life, good oxygen-carrying capacity and tissue oxygen delivery, few side effects, and reasonable cost. Their use should not interfere with diagnostic tests or the clinical diagnosis of serious disease processes (Table 1).

Table 1. Desirable Characteristics of a Blood Substitute

- Long circulation half-life
- Stability (oxidation, metabolism)
- Long shelf life
- Favorable colloid oncotic pressure
- Good O₂ transport
- Oxygen delivered to tissue in need
- Few side effects
- No interference with laboratory tests
- Reasonable cost

Hemoglobin-Based Oxygen Carriers

Structure and Design

Free, unmodified human tetrameric hemoglobin rapidly dissociates into dimers and monomers when removed from its normal environment inside the erythrocyte. Dissociation into hemoglobin fragments leads to renal toxicity and greatly increases oxygen affinity, precluding effective tissue oxygen delivery.

Manufacturers of HBOCs therefore have employed strategies modifying the native hemoglobin molecule to stabilize it, extend intravascular residence time, and return its oxygen-unloading properties into the range of erythrocyte-based hemoglobin. One such method is intramolecular cross-linking between alpha and beta chains. Other methods involve polymerization, pyridoxylation, or conjugation to larger molecules, such as albumin or polyethylene glycol ("pegylation"). Encapsulation of hemoglobin into a liposome or polymer structure has also been pursued. There is a dilemma in the trade-off between desirable properties: Larger hemoglobins and liposomes may have longer half-lives and are less active in scavenging nitric oxide (NO) from the endothelium (which limits their hypertensive properties). Unfortunately, larger molecules also can be more antigenic and can undergo accelerated auto-oxidation, hemoglobin peroxidation, and heme loss.⁵ Smaller species are less antigenic, are filtered by the kidneys, are more oncologically active, and have shorter vascular residence times.

Such "designer" modifications stabilize the molecule's tetrameric structure and affect molecular size, renal filtration, P50 (defined as the oxygen tension at which hemoglobin oxygen saturation is 50%), affinity to NO binding, circulation half-life, viscosity, and colloid oncotic pressure. The raw material for hemoglobin solutions can be human red blood cells, bovine red blood cells, or recombinant bacterial sources. To date, no

hemoglobin solution has been approved for human use, except Oxyglobin (Biopure Corp., Cambridge, MA) in South Africa. Several HBOCs, however, are still being investigated for safety and efficacy in humans (Table 2).

Properties

Although there are product-specific variations, the P50s of HBOC solutions are generally similar to those of fresh blood but higher than those of stored blood. One notable exception is MalPEG-Hb (polyethylene glycol-modified human hemoglobin; MP4), whose P50 is 5 to 6 mm Hg. The molecule was designed to achieve tight oxygen binding so as not to release oxygen too early and thereby cause precapillary vasoconstriction.⁶ In fact, Tsai et al⁷ confirmed in an animal microcirculatory model that MP4 preferentially induces oxygen release from both red blood cells and plasma hemoglobin in *capillaries*, compared with preferential release at the *precapillary* level with polymerized bovine hemoglobin; furthermore, MP4 induced vasodilation in precapillary arterioles, compared with vasoconstriction induced by alpha-alpha cross-linked hemoglobin.⁸ Circulation half-lives are measured in hours (4 to 24 hours, often dose-dependent) rather than days, as would be the case for red blood cells.

Many HBOCs were known to elevate systemic and pulmonary vascular resistance, resulting in a mild reduction in cardiac index. For example, the now-abandoned diaspirin cross-linked hemoglobin (DCLHb; Baxter Healthcare Corp., Deerfield, IL), an alpha-alpha cross-linked tetramer, produces a predictable and sustained increase in mean arterial pressure (MAP) and in systemic and pulmonary vascular resistance.⁹ At the microcirculatory level, functional capillary density is reduced.¹⁰ The pressor response is dose-dependent and pharmacologically reversible and exhibits a "ceiling effect."^{11,12} In human volunteers, 100 mg/kg of DCLHb increased median systolic blood pressure maximally by ≤ 10 mm Hg and diastolic blood

Table 2. Current Oxygen Carriers

HBOC (Manufacturer)	Raw Materials	Structure for Stabilization	Hemoglobin Concentration (g/dL)	Size (kD)	T _{1/2} (h)	Oncotic Pressure (mm Hg)	Viscosity vs. Blood (%)	P50* (mm Hg)
Hemopure [HBOC-201; Oxyglobin†] (Biopure Corp., Cambridge, MA)	Bovine RBC	Glutaraldehyde-polymerized	13	130–500	8–17‡	17	30	34
Polyheme (Northfield Laboratories Inc., Evanston, IL)	Human RBC	Polymerized Hb; pyridoxylated 2,3-DPG site	10	>126	24	20–25	30–40	28–30
Hemospan [MalPEG-Hb; MP-4] (Sangart Inc., San Diego, CA)	Human RBC	Polyethylene glycol	4.2	90	18	49	55	5–6
Oxygent [Perflubron + perflubrodec] (Alliance)§	Perfluorochemical; egg-yolk phosphor-lipid	Perfluorodecyl bromide	0	0.18 μ m diameter	12	NA	100	NA

*Normal human P50 = 28 mm Hg.

†Food and Drug Administration-approved for veterinary use; limited approval for human use in South Africa.

‡Dose = 0.2–0.6 g/kg.

§Currently only considered for transplant organ-preservation applications.

pressure by no more than about 15 mm Hg.¹³ Biopure's HBOC-201 increased MAP by about 10 mm Hg when a dose of 0.6 g/kg was administered to healthy volunteers,¹⁴ but it had no significant effect on blood pressure when given to surgical patients.¹⁵

In the author's clinical investigative experience with 1 g/kg DCLHb administered to patients undergoing major orthopaedic and urologic surgery, MAP was increased by an average of about 20 mm Hg with the hypertensive effect persisting for 24 to 30 hours after administration.¹⁶ Although HBOC-associated hypertension has not been associated with adverse cardiac events, selected patients required pharmacologic treatment. It should also be noted that DCLHb administration to U.S. trauma victims was associated with more than twice the mortality compared with controls.¹⁷ It has been hypothesized that this may have been the result of vasoconstrictive tissue hypoxia aggravating serious blood loss.¹⁸

Two HBOCs still in clinical trials, MalPEG-Hb¹⁹ and PolyHeme (Northfield Laboratories Inc., Evanston, IL),²⁰ appear to have minimal if any vasoconstrictive effects. Nevertheless, MalPEG Hb has been shown to result in fewer hypotensive episodes after spinal anesthesia compared with crystalloid solution.²¹

Although originally the mechanisms thought to account for this pressor effect are the scavenging of NO from vascular endothelium, other possibilities exist, including facilitation of endothelin production and a sympathomimetic effect. The smaller the hemoglobin molecule, the more effectively it interacts with the endothelium, penetrating it and scavenging endothelial NO to form met-hemoglobin and NO-hemoglobin.²² An alternative explanation for the vasoconstrictive properties of HBOCs with near-normal P50 values is that these HBOCs give off oxygen too early, that is, at the precapillary level, thus inducing oxygen-mediated "autoregulatory" vasoconstriction.⁶

In the operative setting, several factors may blunt HBOC-associated hypertensive tendencies. The hypotensive action of surgical hemorrhage,²³ volume depletion,^{23,24} and anesthetic effects^{21,25} may diminish HBOC-related blood pressure enhancement.

Hemoglobin solutions have colloidal properties (Table 2), are highly purified, generally do not affect coagulation, and are only weakly antigenic. Modified molecular hemoglobin undergoes oxidation to methemoglobin and leaves the circulation primarily through the reticuloendothelial system. Preclinical and clinical studies indicate that modified hemoglobins can mildly increase the concentrations of plasma creatine phosphokinase (but not MB fraction), hepatic enzymes, reticulocyte count, bilirubin, and amylase.²⁶⁻²⁸ In a study of patients undergoing high blood-loss (approximately half of an adult's blood volume) surgical procedures, 1 g/kg DCLHb was associated with transient increases in serum lactate dehydrogenase, aspartate transaminase, total bilirubin, creatine kinase, blood urea nitrogen, and amylase. A high incidence of yellow skin discoloration and asymptomatic hemoglobinuria were also present.¹⁶

Gastrointestinal side effects include flatulence, abdominal pain, nausea, vomiting,^{13,14,29} and, possibly, pancreatitis. DCLHb, HBOC-201, as well as PolyHeme28 have been associated with elevations in serum amylase and bilirubin, and HBOC and DCLHb with elevations in lipase.^{30,31} Although pancreatitis has been observed after perioperative administration of HBOC,³⁰ confounding surgical effects could not be excluded as pancreatitis occurs frequently after major abdominal surgery.³²⁻³⁴ Still, heme-pocket modification of a cross-linked hemoglobin to reduce NO scavenging resulted in improved restoration of the pancreatic microcirculation after hemorrhagic shock.³⁵ The gastrointestinal side effects of DCLHb and HBOC-201 may be related to its ability to interfere with NO production and signaling,³⁶ possibly causing pancreatic ischemia as well as affecting

gastrointestinal and biliary motility. Judging from preclinical studies of intestinal and portal system blood flow after administration of DCLHb,^{37,38} gastrointestinal side effects are unlikely the result of tissue ischemia.

Toxicity

Toxicity of hemoglobin solutions has historically been related to impurities such as red blood cell membrane residues, endotoxin, free dimers, and monomers. With vastly improved purification procedures, concern over toxicity from impurities is waning. In particular, the issue of renal toxicity has been overcome. In rats, 0.4 g/kg DCLHb did not affect renal blood flow.³⁸ Creatinine clearance was neither decreased by 0.1 g/kg DCLHb⁶ nor by 0.32 g of recombinant hemoglobin³⁹ in human volunteers. It was similarly unaffected by up to 0.7 g/kg in critically ill patients with sepsis syndrome⁴⁰ by 750 mL 10% DCLHb in cardiac patients,⁴¹ and by 1.0 g/kg DCLHb in patients undergoing high blood-loss surgery, despite the occurrence of hemoglobinuria at the higher doses.¹⁶ Neither was renal toxicity observed with HBOC-20142 or polymerized hemoglobin.⁴³

Free hemoglobin, when directly applied to central nervous system tissue, is neurotoxic. It stimulates leukocyte migration and vascular adherence. Hemoglobin also activates platelets, promoting aggregation.⁴⁴ Circulating ferrous hemoglobin, even when highly purified, undergoes a number of reactions that may contribute to toxicity.⁴⁵ Ferrous hemoglobin binds NO about 3,000 times more tightly than carbon monoxide and therefore effectively removes any NO in its vicinity, accounting for the vasoactive properties. Free hemoglobin is converted to methemoglobin at a rate as fast as 4% per hour; this reaction can lead to the generation of free radicals. Hemoglobin also has a number of "pseudoenzymatic" properties, which could lead to oxygenation, lipid peroxidation, and cytotoxicity. Further possibilities for toxicity arise from the degradation products of hemoglobin's heme moiety such as hemin. Red blood cells contain antioxidant enzymes such as catalase and superoxide dismutase, which may help limit ischemia reperfusion injury. It has been speculated that the administration of pure hemoglobin (i.e., without antioxidants) may lead to a potentially higher risk of reperfusion injury.⁴⁶ Efforts are being made to develop poly-hemoglobin-enzyme complexes (such as with catalase or superoxide dismutase) which, in theory, would serve to help limit oxidative toxicities of hemoglobin.⁴⁷

Although many early trials indicate that some HBOCs have not been associated with severe toxicity, more study in clinical situations is required before their side effects will be fully understood. Investigation into the effects of HBOCs on the gastrointestinal system, pulmonary vasculature, and organ function during hemorrhagic and other stress is particularly needed. Furthermore, the characteristics of HBOC-assisted oxygen delivery and tissue oxygen availability during supply-dependent conditions require additional investigation.

Perfluorocarbons

Perfluorocarbons (PFCs) are inert aromatic or aliphatic chemicals that can dissolve oxygen and carry it in solution throughout the body. They typically carry 4 to 50 volume % at an arterial oxygen pressure of 160 mm Hg. Their ability to carry oxygen is directly proportional to their concentration in blood and, importantly, to the partial pressure of oxygen. The first fluorocarbon to be approved for clinical use (during percutaneous transluminal coronary angioplasty) was Fluosol DA-20, which contains 20% emulsified fluorocarbon. When used as an oxygen-carrying volume

expander, Fluosol DA was associated with a number of limitations, including low oxygen-carrying capacity, short shelf-life, temperature instability, and serious side effects, prompting its withdrawal from the market. Second-generation PFCs, such as perfluoro-octylbromide and perflourodecyl bromide formulated in a lecithin-based emulsion (perflubron, Oxygent; Alliance Pharmaceuticals, San Diego, CA), were investigated extensively. They showed promise because of a much higher oxygen-carrying capacity, a much improved shelf-life, low viscosity, and less interference with normal pulmonary surfactant mechanisms.⁴⁸ Spahn et al⁴⁹ reported that a perflubron emulsion combined with 100% oxygen ventilation reversed transfusion-predetermined triggers such as hypotension, tachycardia, high cardiac output, and low venous oxygen saturation in orthopaedic surgical patients.

Perflubron emulsion also reduced the need for allogeneic red blood cell transfusion in major noncardiac surgery when used in conjunction with acute normovolemic hemodilution.⁵⁰ In experimental hemorrhagic shock, perflubron emulsion restored hepatic microcirculatory flow and oxygenation while supporting blood pressure.^{51,52} After voluntary suspension (for a greater incidence of stroke in the Oxygent treatment group)⁵³ of a cardiac surgery trial of perflubron emulsion in 2001, the future of this oxygen carrier has become uncertain.

Because PFCs are not metabolized, but excreted unchanged via the lungs, their potential for cytotoxicity is thought to be limited. There is no antigenicity. However, because PFCs are taken up avidly by the reticuloendothelial system, they increase liver enzymes and result in hepatosplenomegaly. Because of the extensive uptake in the reticuloendothelial system and impairment of neutrophil function, they may interfere with host-defense mechanisms. Monocyte and macrophage activation may lead to release of prostaglandins, endoperoxides, and cytokines, which probably accounts for the symptoms of flushing, backache, fever, chills, headaches, and nausea observed in clinical trials. Platelet count decreases by as much as 40% because of increased platelet clearance from PFC-induced modification of platelet surfaces.⁵⁴ PFCs also may prolong the actions of certain drugs, including barbiturates.

Potential Clinical Uses and Effectiveness of HBOCs
Major Surgical Bleeding and Hemorrhagic Shock

Fluid therapy for the acutely bleeding patient can be accomplished initially with either crystalloid or colloid solutions. Blood transfusion is begun when, despite volume resuscitation with non-oxygen-carrying solutions, there is concern about tissue ischemia and resultant organ dysfunction. Accumulation of base deficit and serum lactate and low central venous oxygen concentration are all indices of tissue ischemia, which should be taken into consideration in the transfusion decision.⁵⁵ Alternatively, blood is transfused when organ ischemia can be anticipated, given the extent and rapidity of ongoing bleeding.

Hemoglobin solutions are as effective as whole blood in restoring MAP in animals⁵⁶⁻⁵⁸ and humans.²⁴ However, hemoglobins do not match whole blood in its capacity to restore microcirculatory flow and tissue oxygenation. They are, however, more effective than non-oxygen-carrying crystalloid or colloid solutions. In contrast to typical catecholamines, the pressor response of some HBOCs is associated with increased tissue perfusion (DCLHb) and less acidosis (MP-4) in both top-load and hemorrhagic, hypovolemic, animal models.⁵⁸⁻⁶² DCLHb, compared with non-oxygen-containing crystalloid or colloid solutions, resulted in substantially better survival from experimental hemorrhagic shock.^{59,60} This salutary

effect may be related to DCLHb's effect on tissue perfusion and peripheral oxygenation. For example, tissue oxygenation, measured directly by a fluorescence-quenching optode, was restored more effectively in a rat hemorrhagic shock model treated with DCLHb compared with lactated Ringer's solution and albumin.⁶³ Despite increased total peripheral vascular resistance, rat coronary blood flow³⁸ was augmented after DCLHb and human cerebral blood flow was unchanged after infusion of polymerized hemoglobin.⁶⁴

Despite the vasoconstrictive properties of some HBOCs, tissue hypoperfusion may be counteracted with added blood oxygen-carrying capacity and better rheologic properties. In spontaneously hypertensive rats subjected to middle cerebral artery occlusion, hemodilution with DCLHb resulted in a significant dose-dependent reduction in the extent of brain injury and cerebral edema.⁶⁵ The most effective reductions in ischemic injury occurred in those animals in which the inherent hypertensive response to DCLHb was not inhibited. Second-generation perfluorochemicals have also been shown to decrease cerebral ischemic injury.⁶⁶

The effect of HBOCs on cardiac index is more controversial, with some studies reporting a slight decrease,⁶⁷ whereas others report no change.⁴⁰ Calculated oxygen delivery generally follows cardiac output, thus accounting for the slight decreases reported in the former. However, increased tissue oxygen-diffusing capacity has been shown.¹⁴ Furthermore, the equivalent or enhanced oxygen-unloading capacity of HBOCs compared with blood should allow favorable tissue oxygen delivery, or at least counteract vasoconstrictive effects of free hemoglobin. It is therefore understandable why their use in trauma patients and in those with substantial surgical bleeding has been proposed. Table 3 suggests potential uses of HBOCs for therapy of patients suffering large blood losses.

Of the artificial hemoglobin preparations still under active clinical investigation, only PolyHeme has been studied in over 250 acute trauma and emergency surgery patients. Up to 20 units (or 1,000 g) PolyHeme have been administered with few side effects and no obvious hypertension.⁶⁸ After demonstrating that extreme hemodilution in primates was well tolerated, with preservation of oxygen consumption,⁶⁹ it was gratifying to see that mortality in

Table 3. Potential Use of Hemoglobin-Based Oxygen Carriers in Patients with High Blood Loss

<p>Emergency Administration</p> <ul style="list-style-type: none"> • Trauma, especially penetrating • Unexpected surgical bleeding • Unexpected bleeding from disease (e.g., gastrointestinal tract) • Difficult cross-match <p>Elective Administration</p> <ul style="list-style-type: none"> • Acute normovolemic hemodilution* • Acute hypervolemic hemodilution* • Replacing blood transfusion during expected active surgical bleeding • Replacing blood transfusion postoperatively <p>Other Potential Applications</p> <ul style="list-style-type: none"> • Organ preservation • Cardioplegia augmentation • Ischemic rescue • Sensitization of tumor bed to radiotherapy

*Especially in patients presenting with low initial hematocrit.

PolyHeme-treated trauma patients with extremely low red blood cell hemoglobin concentrations (<3 g/dL) was low.⁶⁸ It is conceivable that this favorable safety and efficacy profile of PolyHeme results from its lack of tissue vasoconstriction as well as from other positive effects such as abrogation of neutrophil priming, a putative risk factor in multiorgan failure.⁷⁰ At this point, PolyHeme holds the best immediate promise for becoming available as an HBOC useful for resuscitation from hemorrhagic shock. Nevertheless, the pivotal phase III PolyHeme trauma trial has been completed without showing either a benefit in mortality or noninferiority compared to blood.

MalPEG-Hb demonstrates theoretical promise for such an indication as well. MP-4's favorable capillary oxygen unloading properties, lack of vasoconstriction, facilitated oxygen diffusion characteristics, and colloidal properties should combine to yield an effective resuscitation fluid in rapidly bleeding patients. Indeed, during severe experimental hemorrhage and hemodilution, MP-4 restored functional capillary density, acid base status, and tissue oxygenation better than hydroxyethyl starch, stroma-free hemoglobin, and bovine hemoglobin, but not autologous shed blood.^{7,62,71,72} Bovine hemoglobin (Oxyglobin), while returning blood pressure, heart rate, and central venous pressure to normal after hemorrhage, failed to correct oxygen delivery and cardiac index.⁷³ Bovine hemoglobin, if approved for human use in the United States, therefore may not represent as desirable a therapeutic in hemorrhagic situations as PolyHeme. Bovine hemoglobin (Oxyglobin) is approved for the indication of acute anemia in South Africa, where contaminated blood supply is frequently an issue in transfusion practice. Oxyglobin is also sold for veterinary use.

Clinical Efficacy in Blood Sparing

Because of their short half-lives, blood substitutes are likely to be used primarily as a “bridge to transfusion.” For example, the half-life of DCLHb administered to patients undergoing high-blood-loss surgery was approximately 10 hours.⁷⁴ Administration of DCLHb after bypass spared nearly 20% of cardiac patients from allogeneic transfusion.⁶⁷ With a polymerized hemoglobin, 34% of patients were spared allogeneic transfusion.⁷⁵ Perflubron emulsion in conjunction with acute normovolemic hemodilution resulted in one- to two-unit reductions in red blood cell transfusion.⁵⁰ Still, there are concerns that the administration of artificial oxygen carriers merely delays blood transfusion rather than truly substituting for it.

Preoperative acute normovolemic hemodilution may become

more attractive with the use of modified hemoglobins or PFCs as diluents. The short half-life of HBOCs does not present a significant liability for this clinical application. Volume replacement with HBOCs (compared with crystalloid or colloid) during acute normovolemic hemodilution for autologous collection might result in a greater yield of perused blood components. Furthermore, patients with low preoperative hematocrit might receive an infusion of HBOC to “tide them over” a limited period of intraoperative or postoperative bleeding, after which autologous or allogeneic blood would be administered if still needed.

Caveats Regarding the Use of HBOCs for Major Blood Loss

The safety (see Tables 4 and 5) of large-scale and rapid transfusion of HBOCs in human traumatic injury remains to be shown in a large scale randomized controlled trial. Although several small series of patients undergoing high blood-loss elective surgery tolerated oxygen carriers relatively well,^{16,50,68} pivotal phase III trials have been halted because of an imbalance of severe side effects. A phase III cardiac study of perflubron emulsion was stopped because of an apparent imbalance in neurological events (stroke)¹⁸ and a trial of DCLHb for resuscitation of trauma patients was halted because of higher mortality compared to controls.¹⁷

Because HBOCs are associated with systemic hypertension, concern has been raised over a potential for increased blood loss in hemorrhage. This concern could not be corroborated in preclinical⁶¹ or clinical studies¹⁶ conducted in a setting of hemorrhage, but the issue has yet to be clarified in the setting of penetrating trauma.

The clinical use of HBOCs with relatively short half-lives must take into account their tendency for transvascular migration and their rapid clearance through the reticuloendothelial system. Although the initial effect of transfusion may be an immediate increase in vascular volume (enhanced by some HBOCs’ colloidal properties) and blood pressure (mediated by some HBOCs’ vasoconstrictive effect), rapid dissipation of the HBOC requires careful and frequent monitoring of circulatory adequacy, because hypovolemia may re-manifest rather quickly.⁴⁴ Administration of HBOCs whose colloid oncotic pressure is high likely expands blood volume disproportionately,⁷⁶ presumably by recruiting extravascular fluid into the vascular compartment.

At least within the first 24 to 36 hours of administration, free hemoglobins can interfere with the photospectrometric methods used in a variety of clinical laboratory tests. Interference with laboratory testing constitutes an important limitation for the potential clinical use of artificial hemoglobin species.

Table 4. Caveats and Potential Remedies in the Clinical Use of Hemoglobin-Based Oxygen Carriers

Caveats	Remedies
Hypertensive tendency; cardiac afterload stress	Coadministration of nitroglycerin, other vasodilator
Pulmonary hypertension, right ventricular dysfunction	Coadministration of pulmonary vasodilator
Short intravascular residence time; recurrence of hypovolemia	More frequent assessment and adjustment of intravascular volume
Volume overload, particularly with high-oncotic pressure solutions	Close monitoring of indices of vascular volume
Interference with diagnostic blood tests	Avoidance of photospectrometric methods; removal of free hemoglobin from specimens; other correction algorithms
Hemoglobinuria interfering with diagnosis of transfusion reaction	Special prearranged testing protocol
Immune depression as larger Hb species overwhelm the reticuloendothelial system	Unknown
Possible NO-related gastrointestinal or other organ injury	Co-supply NO donor or precursor; redesign molecule

Hb, hemoglobin; NO, nitric oxide.

Table 5. Side Effects and Toxicity of Current Hemoglobin Oxygen Carriers

HBOC (Manufacturer)	Interference with Diagnostic Tests	Laboratory Test Abnormalities	Nonserious Side Effects	Serious Adverse Events
Hemopure [HBOC-201; Oxyglobin*] (Biopure Corp., Cambridge, MA)	PT,† PTT,† fibrinogen,† total protein, albumen, alkaline phosphatase, LDH, AST, ALT, GGT, amylase, lipase, cholesterol, lactate	Increased iron, ferritin, erythropoietin;	GI discomfort, N/V, dysphagia, fever, jaundice, hypertension	Myocardial infarct
Polyheme (Northfield Laboratories, Evanston, IL)	No information	No information	Hypertension, rash, kidney or liver damage‡	Cardiovascular ⁷⁷
Hemospan [MalPEG-Hb; MP-4] (Sangart Inc., San Diego, CA)	None reported	Mildly increased lipase, amylase	GI symptoms, mild BP elevation; brady-dysrhythmias	None so far
Oxygent [Perflubron + perflubrodec] (Alliance Pharmaceuticals, San Diego, CA)	Spurious elevation in platelet counts*; interferes with hemolyzing Co-oximeters	Transient thrombocytopenia	Febrile response	Postoperative ileus; chest tube bleeding; stroke

*Automated cell counters only.

†Optical method only.

‡www.uuhsc.utah.edu/polyheme/faq.htm#risks.

PT, prothrombin time; PTT, partial thromboplastin time; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; GI, gastrointestinal; N/V, nausea and vomiting; BP, blood pressure.

Summary

The field of viable artificial oxygen carriers has narrowed considerably. A new HBOC (MalPEG-Hb) has been developed based on recent insights into microcirculatory oxygen unloading. Of the HBOCs left standing, PolyHeme appears to hold the most immediate promise for clinical use in high blood-loss situations, although the bovine hemoglobin Oxyglobin is the first to be approved for commercial veterinary use and for human use in South Africa. Still, much work remains to be accomplished to document the safety of large-scale blood replacement with artificial oxygen carriers in humans.

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