

THE SEARCH FOR A BLOOD SUBSTITUTE

The quest for a blood substitute dates back to ancient times when mixtures of wine, water, and honey were infused intravenously. Now that we recognize that the animating principle that gives blood its life-restoring properties is its ability to deliver oxygen, the search has focused on oxygen carriers. Although we commonly call these oxygen therapeutic agents “blood substitutes” or “red cell substitutes,” they share a limited number of characteristics with blood and are intended to serve the single function of gas exchange.

Two primary motivations have inspired renewed interest in the development of red blood cell (RBC) substitutes over the last 20 years: concerns about the infectious risks of transfusion and adequacy of the blood supply. An acellular substitute with permissive storage requirements and universal compatibility would be of great use on the battlefield or at accident scenes, where transfusion of banked red blood cells is not possible.

The two classes of materials that have been studied most extensively as potential blood substitutes are perfluorocarbon emulsions and modified hemoglobin solutions.^{1,2} Liposomes containing hemoglobin have also been studied but have yet to reach clinical testing in humans and will not be discussed here.

Perfluorocarbons. Perfluorocarbons are a class of compounds consisting of carbon backbones that have been extensively substituted with fluorine. Perfluorocarbons also have high solubility for respiratory gases such as O₂ and CO₂. Because it is necessary to expose perfluorocarbons to very high ambient PO₂ levels in order to load them with physiologically-useful amounts of O₂, patients must be on supplemental O₂. In addition, perfluorocarbons are almost completely immiscible in water—so they must be prepared as emulsions for most biological applications.

The first perfluorocarbon emulsion that was tested clinically as a blood substitute was Fluosol-DA. However, in a phase II clinical trial in hemorrhaging patients who refused blood transfusions for religious reasons, Fluosol-DA did not improve mortality.³

The disappointing Fluosol-DA results pointed the way to developing two new perfluorocarbon emulsions with higher O₂-carrying capacity: Oxygent (Alliance) and Oxyfluor (HemaGen). Oxygent's primary transfusion application was in acute normovolemic hemodilution. Replacing blood withdrawn at the beginning of a procedure with an oxygen-carrying solution should permit a more extreme degree of hemodilution and enhance the technique's efficacy. This was borne out in phase II clinical trials,⁴ but phase III clinical trials were discontinued for safety concerns. HemaGen ceased active investigation of Oxyfluor in the mid 1990s.⁵ At present, there are no perfluorocarbons in clinical trials.

Hemoglobin-Based Oxygen Carriers. The other major approach to developing a blood substitute has been the use of modified hemoglobin solutions. Hemoglobin has a number of advantages as an oxygen carrier, including its high capacity for O₂, the absence of red blood cell antigens (once purified), a prolonged shelf life (relative to banked red blood cells), and the ability to withstand rigorous purification and viral inactivation procedures. However, cell-free hemoglobin has significant toxicities—hence the need to modify hemoglobin for use as a therapeutic agent.

SUMMARY

- **The two major classes of materials that have been studied in clinical trials as candidate RBC substitutes are perfluorocarbon emulsions and modified hemoglobin.**
- **Perfluorocarbon emulsions have been studied in transfusion and acute normovolemic hemodilution—and as oxygen carriers in percutaneous transluminal coronary angioplasty and partial liquid ventilation.**
- **Cell-free hemoglobin must be modified to minimize rapid clearance, renal toxicity, and vasoactivity.**
- **Although many candidate RBC substitutes have been studied, to date none has been licensed for human use in the US.**

Cell-free hemoglobin in the bloodstream rapidly dissociates into $\alpha\beta$ dimers that are quickly filtered out of the circulation by the kidney. The rapid loss of intravascular hemoglobin is complicated by toxic effects on the renal tubular endothelium. Therefore, all hemoglobin-based oxygen carriers (HBOCs) under investigation have been modified to prevent rapid clearance and renal toxicity. These modifications have included: cross-linking of the two $\alpha\beta$ dimers to create a stabilized hemoglobin tetramer; polymerization of hemoglobin tetramers to create higher-order n -mers; and surface conjugation with long-chain molecules such as polyethylene glycol, which increases the molecular radius as well as weight of the hemoglobin tetramer. All three techniques have increased the plasma half-life of these modified hemoglobins to the 18-36 hour range and have virtually eliminated renal toxicity.

A much more vexing problem is the vasoactivity of cell-free hemoglobin. Hemoglobin is known to bind nitric oxide (NO) avidly so early on, it was posited that HBOCs, especially those that were smaller (*e.g.* stabilized tetramers), could extravasate into spaces between cells and bind NO.⁶ More recently, it was suggested that HBOCs' pressor effect occurs because most have lower viscosity than whole blood. This reduces the shear stress sensed by the vascular endothelium, which then curtails the production of NO and results in vasoconstriction.⁷ New insights into the regulation of blood flow in the microcirculation suggest that the pre-capillary arterioles are sensitive to PO₂, and respond to elevations by vasoconstriction. This autoregulatory theory predicts that HBOCs that deliver O₂ more readily release oxygen at the arteriolar level, triggering vasoconstriction. In contrast, HBOCs that bind O₂ more tightly (low P₅₀) and have a low diffusion constant for O₂ (*e.g.* due to a large molecular radius) would release O₂ later, *i.e.* in the capillary bed instead in the arteries. It appears, however, that the P₅₀ makes only a minor contribution to oxygen delivery; the impact of viscosity remains controversial; and a low diffusion constant appears to be a significant factor.⁹

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Table 1. Hemoglobin-Based Oxygen Carriers in Clinical Trials

Product (manufacturer)	Hemoglobin Source (modification)	Trial Level	Application
PolyHeme (Northfield)	Human (polymerized)	Phase III	Trauma, surgery, ambulance trial
HemAssist (Baxter)	Human (cross-linked)	Phase III Phase II	Trauma, stroke (discontinued) Septic shock, hemorrhagic shock, hemodialysis, CP bypass
Hemolink (Hemosol)	Human (polymerized)	Phase III Phase II	Cardiac surgery (trial suspended) Orthopedic surgery, hemodialysis, CP bypass, ANH
PHP (Ajinomoto/Apex)	Human (conjugated)	Phase III	Septic shock
Hemospan (Sangart)	Human (conjugated)	Phase Ib/II	Orthopedic surgery
PEG Hemoglobin (Enzon)	Bovine (conjugated)	Phase Ib	Radiosensitizer, solid tumors
Hemopure (Biopure)	Bovine (polymerized)	Phase III Phase II Phase II Pre-clinical	Surgery – orthopedic, cardiac Sickle cell crisis, surgery, trauma, ANH Angioplasty and stents Erythropoiesis
Optro HemAssist (Somatogen/Baxter)	Recombinant (cross-linked)	Phase III Phase II Phase I	Severe traumatic shock (discontinued) ANH, surgery Erythropoiesis in ESRD, refractory anemia

Abbreviations: ANH – acute normovolemic hemodilution; ESRD – end stage renal disease; CP Bypass – cardiopulmonary bypass

These considerations have directed the research into designing a clinically effective HBOC. Eight companies have brought HBOCs to the clinical trial stage of development (Table 1). Two have been studied primarily for applications other than transfusion (e.g., radiosensitization in solid tumors and treatment of hypotension in septic shock). Studies with HemAssist (Baxter) and Optro (Somatogen, owned by Baxter) were terminated after unfavorable outcomes in two phase III studies.¹⁰

Northfield completed a phase III trauma trial of PolyHeme¹¹ in which trauma patients receiving the hemoglobin solution needed fewer blood transfusions and currently is in a phase III ambulance trial designed to evaluate efficiency in severely injured and bleeding patients beginning at the trauma site continuing until 12 hours after injury. This study is being conducted under an exception to federal rules requiring informed consent that allows research in certain emergency, life-threatening situations.

Biopure studied Hemopure in acutely anemic adult patients undergoing orthopedic surgery.¹² The results of the trial, completed in 2000, raised concerns because of adverse events. As a result, the Food and Drug Administration required additional preclinical animal studies prior to approving further U.S. clinical trials. A phase II trial is on-going in Europe to access the safety of low-dose Hemopure as a cardioprotective agent in patients undergoing angioplasty and stent procedures. The trial outcome addresses tissue oxygen status rather than anemia. Hemosol suspended a phase III trial of Hemolink in surgery, citing possible safety problems.

A newer product, Hemospan (Sangart) has been designed to minimize autoregulatory effects and features a low P₅₀. Six strands of polyethylene glycol are attached to the hemoglobin molecule. This results in a larger molecular radius, hence high viscosity and low diffusion coefficient.¹³ A recently reported phase Ib/II clinical trial conducted in Sweden in orthopedic surgical patients found no adverse events attributed to Hemospan.

Of note, hemoglobin solutions interfere with some colormetric laboratory tests, such as PT PTT.

Although the transition from lab to clinic has been difficult, these candidate blood substitutes have taught us much about the delivery of oxygen and its regulation.

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