

Blood substitutes as pharmacotherapies in clinical practice

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Purpose of review

To discuss the development and current status of blood substitutes, including hemoglobin-based oxygen carriers (HBOCs) and perfluorocarbons. Research in this field offers an important view into the future of transfusion medicine in the operating room, as well as in trauma and combat arenas.

Recent findings

A pivotal multinational phase III trial of the Biopure product HBOC-201 (Hemopure) has been completed in orthopedic surgery patients. HBOC-201 consists of polymerized bovine hemoglobin and has already been well tolerated in patients undergoing cardiopulmonary bypass and abdominal aortic reconstruction. Polyheme is a polymerized human hemoglobin in early phase III clinical trials with trauma patients, having infused up to 10 000 ml, with efficacy apparently demonstrated in phase II. The Sangart product, Hemospan, is currently undergoing phase II trials.

Summary

Polymerized hemoglobin preparations have proven most successful in clinical trials due to their improved side-effect profile. The goal is to evaluate blood substitutes with enhanced intravascular retention, reduced osmotic activity, and attenuated hemodynamic derangements such as vasoconstriction. Although not without substantial morbidity and mortality, the current safety of allogeneic blood transfusion demands that comparative studies show minimal adverse effects, as well as efficacy and potential for novel applications.

Keywords

blood substitutes, hemoglobin-based oxygen carriers, oxygen therapeutics, perfluorocarbons

Introduction

This article discusses the development and current status of blood substitutes, including hemoglobin-based oxygen carriers (HBOCs) and perfluorocarbons. The article includes a discussion of risks of blood transfusion, an overview of an ideal blood substitute, a brief history of HBOCs and perfluorocarbons, physiology of oxygen carrying, adverse effects of HBOCs and perfluorocarbons, current products in clinical trials, and the outlook of blood substitutes as pharmacotherapies for the future. In an earlier review, Jahr *et al.* elaborated on these issues [1]; this update provides newer information and recent science.

Risks of blood transfusion

Almost concurrent with the development of blood transfusion as a therapy, an alternative has been researched in an effort to circumvent the adverse effects of transfusion [1]. The risks of allogeneic blood transfusions are multiple and include infectious transmission, delayed postoperative wound healing, transfusion reactions, transfusion-related acute lung injury, immunomodulation and potential risk of cancer recurrence [2–4]. In addition, the logistical challenges inherent in blood cross-typing and lack of portability limit use of allogeneic transfusion in trauma and combat casualty care. Blood supply is in crisis with increasing shortages [5]. The issue of the safety of ‘old’ blood-bank blood compared to freshly donated blood-bank blood has also been raised [6,7].

The American Society of Anesthesiologists proposed and published revised guidelines for the intelligent use of blood and blood products [8**]. These guidelines lowered the ‘transfusion trigger’ in many cases and allowed anesthesiologists to become leaders and consultants in blood-saving techniques [9]. Other techniques have been documented as well, including preoperative autologous blood donation, preoperative use of erythropoietin, acute normovolemic hemodilution, perioperative blood salvage/autotransfusion (cell saver), pharmacologic treatment (antifibrinolytics/antiinflammatories/platelet function; use of desmopressin, aminocaproic acid, and aprotinin), anesthetic technique (maintaining normothermia, optimal fluid replacement to maintain blood coagulation, and hyperoxic ventilation or hypotensive anesthesia), surgical technique, accepting minimal hemoglobin levels, transfusion algorithms based on coagulation monitoring, and artificial oxygen carriers/blood substitutes [1,9–11]. These techniques have allowed anesthesiologists responsible for perioperative care to manage transfusion in a more

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Abbreviations

DCLHb diaspirin cross-linked hemoglobin
HBOC hemoglobin-based oxygen carrier

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Table 1 Ideal characteristics of a blood substitute

Nonantigenic
Similar to natural hemoglobin in terms of oxygen and carbon dioxide transport and delivery (This concept has been challenged in new generation HBOCs; see section on outlook of blood substitutes as pharmacotherapies for the future)
Does not cause increases in arterial or pulmonary blood pressure
Sufficient half-life in circulation
Does not form methemoglobin, activate complement, increase white blood cell count, reacts with plasma substitutes or platelets
Absence of renal toxicity
Stable at room temperature
Immediate availability
Easy to administer
Does not overload reticuloendothelial system
Possible long-term storage
Does not cause oxidation and free radical formation

HBOC, hemoglobin-based oxygen carrier. Modified from [1,12*].

reasonable and scientific way. They have also facilitated institutions in developing a global blood conservation plan, including a multidisciplinary approach [9].

Overview of an ideal blood substitute

Current research in blood substitutes has centered on perfluorocarbons and hemoglobin solutions. The ideal characteristics of a blood substitute are shown in Table 1 [1,12*].

Perfluorocarbons are low-molecular-weight linear or cyclic hydrocarbons in which hydrogen atoms of the carbon chain have been substituted by fluoride, leading to total chemical inertness and a complete lack of metabolism *in vivo*. They have a high solubility coefficient for oxygen, higher than the coefficient for oxygen dissolved in plasma oxygen [13].

HBOCs may be manufactured from allogeneic, xenogeneic, or recombinant sources. These sources of hemoglobin are human (obtained from lysis of red blood cells in expired units of donated bank blood), animal (bovine or transgenic swine), and biotechnologic (*Escherichia coli* and *Sarcomyces cerevisiae*).

Hemoglobin molecules extracted from red blood cells are modified by microencapsulation or cross-linkage. This stabilizes the hemoglobin molecules and also allows sterilization of the products to remove microorganisms. The different methods of preparation of modified hemoglobin solutions are shown in Table 2 [14,15].

Brief history of hemoglobin-based oxygen carriers and perfluorocarbons

HBOCs have been studied since 1934, when Amberson purified bovine hemoglobin and administered it to study animals, and 1949, when he purified human hemoglobin and infused it into anemic patients (see extensive discussion in reference [1]). The US Army developed a tetra-

Table 2 Methods of preparation of modified hemoglobin solutions

From natural stoma-free hemoglobin (human, bovine)
Hemoglobin cross-linking: [alpha]-[alpha] and [beta]-[beta]
Binding to macromolecules cross-linked with diaspirin or raffinose
Polymerization with glutaraldehyde
Encapsulation
Microencapsulated haemoglobin
Hemoglobin lipid vesicles
Biodegradable polymer hemoglobin nanocapsules
Genetic engineering
Natural or modified haemoglobin
Bacteria
Yeasts

Modified from [14,15].

meric cross-linked hemoglobin (alpha-alpha cross-linked hemoglobin), which then was produced as the Baxter Corporation product, diaspirin cross-linked hemoglobin (HemeAssist) [16]. This product failed clinical trials because of diminished cellular perfusion and increased morbidity and mortality [16]. In the mid-1980s, a number of companies developed 'second generation' HBOCs, including Biopure Corporation with Oxyglobin, approved by the Food and Drug Administration (FDA) and the European Union for canine anemia in 1997 and 1998, respectively, and Hemoglobin glutamer-201 (bovine), Hemopure, approved by the South African Drug Council for treatment of human anemia in 2001, and Polymerized pyridoxilated hemoglobin, PolyHeme by Northfield Laboratories [1]. To date, there have been a number of studies critically evaluating similar products in animal models [17], and a few clinical trials documenting success in phase 1, 2, and 3 trials [18–20]. In addition, novel uses of HBOCs, such as evaluation of circulating plasma and blood volume, have been documented [21].

Newer strategies are currently being developed with products that alter the earlier generation HBOC characteristics of normal viscosity, lowered hemoglobin (from 10–13 g/dl to 4–6 g/dl) and shift of the oxy-hemoglobin curve to the left from the right (p50 30–40 mmHg to 6 mmHg) (OxyVita, zero link polymerized HBOC, Oxyvita Incorporated, New York, USA, and MP4, Hemo-span, Sangart Incorporated, San Diego, California, USA) [22,23]. These products are still early in development with only one published human phase I study [23], however, and these strategies for improved efficacy and safety remain to be validated and verified independently.

Silicone and fluorocarbon are two synthetic organic materials known for their ability to carry oxygen [24]. In the 1960s, Clark and Gollan [25] demonstrated that mice immersed in oxygenated silicone oil or liquid fluorocarbon could breathe in the liquid. Chang [26] also demonstrated that artificial cells formed from a hybrid of silicone rubber and hemolysate were efficient in carrying and releasing

oxygen, although they did not survive in the circulation for long periods of time. Slovitier and Kamimoto [27] demonstrated that finely emulsified fluorocarbon could maintain rat brain function for several hours. Geyer *et al.* [28] demonstrated that finely emulsified fluorocarbon could replace essentially all the blood of rats, with good recovery and survival. Extensive research was undertaken in Japan by Naito and Yokoyama [29]. This work resulted in the development in 1976 of Fluosol-DA 20. This product was assessed to be suitable for clinical testing and was eventually approved by the US FDA for human use in the 1970s [24]. This product was eventually discontinued and is no longer clinically available.

Physiology of carrying oxygen

Several classifications of products are under clinical development as either potential oxygen therapeutics and/or volume expanders. These products are produced as follows. For the HBOCs, the hemoglobin is separated from either the red blood cells or microorganism. If the source of hemoglobin is animal or human, hemoglobin monomers or dimers result. They are associated with a reduced P50, renal infiltration, and a short plasma half-life. To stabilize the small hemoglobin units, they are modified by either cross-linkage, polymerization, or conjugation [30]. With cross-linked hemoglobin, the tetrameric structure of hemoglobin is maintained by an intramolecular cross-linking between the [alpha]-hemoglobin or [beta]-hemoglobin chains. Polymerized hemoglobin solutions contain either oligomers or cross-linked hemoglobin or polymers of hemoglobin chains [30]. Conjugated hemoglobin is formed by linking free hemoglobin to soluble nonhemoglobin polymers. Encapsulated hemoglobin is formed when hemoglobin and enzymes are encapsulated inside artificial red blood cells with artificial membranes, which are either lipid membranes or made of biodegradable material.

Perfluorocarbons do not have the oxygen-bonding properties of hemoglobin but act as simple solvents. The transport and liberation of gases by perfluorocarbons are based on physical solubility, and the quantity of gas dissolved is linearly related to its partial pressure. The perfluorocarbons are administered as emulsions because they are not hydrosoluble. The first generation of perfluorocarbon emulsion developed was Fluosol-DA 20. The second-generation emulsions are Oxygent (Perflubron; Alliance Pharmaceutical Corporation, San Diego, California, USA) and Oxyfluor (HemoGen, St Louis, Missouri, USA), which use triglyceride and egg yolk lecithin [24]. These new solutions have an enhanced oxygen-carrying capacity and a higher stability. Their administration is not associated with hemodynamic effects, and the products are stable at room temperature for more than 1 year. Oxygent has discontinued clinical trials, however. Third-generation emulsions are currently in early preclinical development [24].

Adverse effects of hemoglobin-based oxygen carriers and perfluorocarbons

Hemoglobin solutions have many adverse effects.

- (1) Vasoactivity: increases in systemic and pulmonary arterial pressure and increases in systemic and pulmonary vascular resistance occur with the use of HBOCs. The underlying mechanism is that hemoglobin, free of red blood cells, leaks through the vascular endothelium and binds to nitric oxide to cause vasospasm. The other mechanism is mediated by the vasoconstrictor peptide, endothelin. Newly discovered allosteric and electric properties of hemoglobin [31] seem to control blood pressure and may facilitate tissue oxygenation.
- (2) Nephrotoxicity: stroma-free hemoglobin resulted in oliguria secondary to acute tubular necrosis. This effect was caused by excessive glomerular filtration of hemoglobin dimers, leading to tubular obstruction. The polymerized or cross-linked HBOCs in current phase III trials do not appear to have this toxicity.
- (3) Interference with macrophage function: hemoglobin may block the mononuclear phagocytic system and interfere with essential functions such as ingestion of bacteria.
- (4) Antigenicity: concerns exist with regard to the production of antibodies to xenogeneic hemoglobin.
- (5) Oxidation on storage: concerns exist with respect to the potential for oxidation during storage and exposure to room air with the resultant production of methemoglobin.
- (6) Activation of complement, kinin, and coagulation: free hemoglobin increases platelet aggregation by nitric oxide scavenging.
- (7) Iron deposition: concerns exist regarding hemochromatosis and iron overload.
- (8) Gastrointestinal distress: clinical findings of abdominal discomfort, pain, nausea, and vomiting have been reported. These findings may be related to nitric oxide binding causing gastrointestinal smooth muscle spasm.
- (9) Neurotoxicity: stroma-free hemoglobin may cause excitatory neurotoxicity, because free hemoglobin may be neurotoxic. This may be important if the blood-brain barrier is disrupted as in traumatic head injury, subarachnoid hemorrhage, or hemorrhagic stroke. Cole *et al.* [32] evaluated the effects of [alpha]-[alpha] cross-linked hemoglobin [diaspirin cross-linked hemoglobin (DCLHb)] in subarachnoid hemorrhage and demonstrated that when injected in the cisterna magna, DCLHb does decrease cerebral blood flow more than an equal volume injection of 'mock cerebrospinal fluid' but less than autologous blood. In contrast, improved neurologic outcome may be seen in patients with stroke who were administered small cross-linked tetramers of hemoglobin

(DCLHb, 16) because DCLHb may enable oxygen delivery, whereas thrombus otherwise hinders the passage of red blood cells.

- (10) Free radicals: it has been demonstrated that stroma-free hemoglobin and its breakdown products, heme and free iron, can contribute to the generation of oxygen-free radicals in tissue.
- (11) Interference with diagnosis of transfusion reaction: this is secondary to hemoglobinuria and the presence of plasma hemoglobin not caused by hemolysis.

One additional area of concern with HBOCs is interference with laboratory values. This topic has been studied in detail and is outside the purview of this review [33,34].

The adverse effects of perfluorocarbons include the following effects:

- (1) Limited shelf life: concerns regarding proper storage and temperature exist with perflubron, a second generation perfluorocarbon, because of coalescence of these emulsions into progressively larger droplets and less surface area through which to diffuse oxygen [30].
- (2) Flu-like symptoms: acute and transient facial flushing and backache may occur during the infusion period [30] with a secondary delayed response of fever and chills.
- (3) Complement and phagocytic activation (not believed to occur with second-generation emulsions): large doses can lead to hepatic engorgement and a temporary impairment of the immune defense mechanism, which is dangerous in the presence of infection. Perfluorocarbons are retained in the reticuloendothelial system, resulting in reticuloendothelial suppression. In addition, decreased platelet counts secondary to increased clearance and impaired neutrophil function have been noted.

Due to the viscosity at high concentrations, the maximum concentration producible is only 20%. As perfluorocarbons may only dissolve oxygen and there is no binding function as with hemoglobin, sufficient oxygen carrying can only occur when the patient is breathing 70–100% oxygen [24].

Perfluorocarbons are rapidly removed from circulation and therefore have a short circulation time, which may be an advantage or disadvantage: minimization of side effects versus short efficacy. Concerns regarding an increased incidence of cerebrovascular events have mandated that phase II clinical trials of perflubron were placed on hold in 2001, and Alliance Pharmaceuticals has ceased operations. (S. Faithful, personal communication).

Current products in clinical trials

Two HBOCs are currently undergoing or have completed FDA phase II trials: Hemopure [HBOC-201, hemoglobin-glutamer 250 (bovine); Biopure Corporation, Cambridge, Massachusetts, USA [35–37]], and PolyHeme (polymerized pyridoxilated hemoglobin; Northfield Laboratories, Chicago, Illinois, USA). The published properties of these products are outlined in Table 3 (adapted from [1,38]).

In the Northfield Laboratories study, the therapeutic benefit of PolyHeme was compared directly with that of allogeneic red blood cells in the treatment of acute blood loss. PolyHeme was able to maintain total hemoglobin concentration and also reduced the amount of donated blood the patient needed by nearly half. Phase III clinical trials are underway, having infused up to 10 000 ml with no reported side effects and with efficacy apparently demonstrated. Northfield Laboratories filed for FDA approval in August 2001 [39]. Recent adverse events in phase III clinical trials have led to press reports (website: <http://www.northfieldlabs.com>).

Biopure Corporation has completed its worldwide pivotal phase 3 clinical trial in human patients on Hemopure. Their veterinary product, Oxyglobin [hemoglobin-glutamer 200 (bovine), HBOC-200], has been approved by the FDA for routine use in canine anemia (see Table 3). In April 2001, Hemopure was approved for use in South Africa for acute anemia in surgery patients, and it is the first HBOC approved for human use. Biopure Corporation has recently described the safety and efficacy results of its phase 3 clinical trials [35–37]. Baxter has discontinued clinical trials on DLCHb (HemeAssist), as has Hemosol

Table 3 Product characteristics

	HES	HBOC-200	OxyVita	MP4	Whole blood	HBOC-201	Poly-Heme
Source	Plant	Bovine	Bovine	Human	Human	Bovine	Human
Avg MW (kDa)	670	200	33 000	90		64–500	150
Hgb (g/dl)	NA	13	6	4.2	13	13	10
P50	NA	34	6.4	6	26	36–38	28–30
Viscosity (cP)	NA	2.8	2.8	2.5	5–10		
Tetramers (%)	NA	<5	<1	100	NA		
Hill coefficient	NA	1.3	1.2	1.2	2.7		
COP (mmHg)	32	42	2.2	55	2	25	20–25
Molecular radius (nm)			25	9.3	5×10^5		

HES = 6% hetastarch in 0.9 sodium chloride injection; HBOC-200 = Hemoglobin glutamer-200 (bovine), Oxyglobin; OxyVita = zero link polymerized HBOC; MP4 = Hemospan; HBOC-201 = Hemoglobin glutamer-201 (bovine), Hemopure; PolyHeme = Polymerized pyridoxilated hemoglobin; MW, molecular weight; COP, colloid oncotic pressure. Adapted from [1,38].

Table 4 The potential uses of hemoglobin-based oxygen carriers

Shock
Organ ischemia
Red blood cell incompatibility
Acute lung injury
Transplant organ preservation
Carioplegia
Sickle cell anemia
Tumor therapy
Air embolism

Incorporated with its product, Hemolink. Sangart Incorporated's Hemospan has completed and published a phase I Trial [23] and has completed a phase II trial (website: <http://www.sangart.com>).

Outlook of blood substitutes as pharmacotherapies for the future

The potential uses of HBOCs in the future are outlined in Table 4. The results of Sangart Pharmaceutical's Hemospan phase II trial have not yet been published, and neither have Biopure Corporation's Hemopure and Northfield Laboratory's PolyHeme phase III studies. Oxyvita is a promising new generation HBOC that is currently undergoing preclinical testing. If safety and efficacy in all these products are validated, FDA approval would be possible. This requires at least 700 subject studies, however. The only completed study that has received FDA review has yet to be approved (Biopure Corporation).

A second generation of the perfluorocarbon emulsion, Fluosol (PHER-O2), developed by Sanguine was FDA approved in 1989 for angioplasty, but was withdrawn in 1994 because of storage issues (website: <http://www.sanguine-corp.com>). Another perfluorocarbon emulsion has been studied (website: <http://www.oxyrase.com/oxyfluor.html>). Finally, Perftran (Moscow, Russia) has developed another perfluorocarbon (perfluorodecalin+perfluoromethylcyclohexylpiperidin), which is permitted for medical application in the Russian Federation (website: <http://www.perftran.ru>).

Conclusion

This article summarizes the current state of development of blood substitutes. It defines the approaches to oxygen carrying and therapeutics. Extensive laboratory study has attempted to define the vasoconstrictive effects of HBOCs compared with the delivery of oxygen, especially in the microcirculation. Novel approaches to the use of HBOCs are described, such as monitoring circulating blood and red cell volume. All the products currently under evaluation are different in their pharmacology and side-effect profile; therefore, no comparisons may be drawn from one to another, because no head-to-head trials have been performed. Two HBOCs are in final stages of evaluation and will likely need to undergo further testing prior to FDA and other regulatory

approval. Several new generation HBOCs are in early preclinical and clinical testing and may provide solutions to older generation HBOCs.

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

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 390).

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