# Intravenous artificial oxygen carriers

*Daniel Farrar, Mike Grocott*

***Haemoglobin-based oxygen carriers and perfluorocarbons have been developed as artificial oxygen carriers which can be safely administered intravenously. Mixed results from clinical trials to date suggest that further work is required to clearly demonstrate clinical efficacy and safety for these exciting products.***

**Dr Daniel Farrar** and **Dr Mike Grocott** are Research Fellows, Centre for Anaesthesia, University College London, London W1T 3AA

*Correspondence to: Dr M Grocott*

Artificial oxygen carriers (AOCs) are synthetic solutions capable of binding, transporting and unloading oxygen within the body. They have been developed as alternatives to blood transfusion, so-called 'bridge oxygenators', or to improve tissue oxygenation of organs with marginal oxygen supply. Other potential uses are listed in *Table 1*.

The modern science of blood transfusion allows rapid provision of whole blood or packed red cells to patients whose oxygen-carrying capacity is thought to be critically impaired. However, although blood transfusion has many benefits, a number of adverse effects are also well recognized. In 1999 the Serious Hazards of Transfusion (SHOT) survey reported 22 deaths and 366 serious events associated with blood transfusion in the UK and Ireland in a 24-month period (Williamson et al, 1999). Adverse outcomes were predominantly associated with patients receiving incorrect blood components. Other risks of blood transfusion include transfusion reactions, transmission of infection (potentially including prion disease), and immunomodulatory effects. In addition, cross-matched blood cannot be immediately available, has a limited shelf life and requires special storage conditions. The availability of blood is decreasing while use continues to rise. These factors have been responsible for driving the development of AOCs.

Two approaches have been used in the development of AOCs: haemoglobin-based oxygen carriers (HBOCs) and perfluorocarbons (PFCs). Haemoglobin-based solutions use free (extra-erythrocytic) haemoglobin (Hb) derived either from red cells or biosynthesized de novo. PFCs are novel compounds capable of dissolving oxygen under physiological conditions and are sufficiently biocompatible to be administered into the intravascular space. The ideal profile of an AOC is described in *Table 2*.

**TABLE 1.**
**Potential applications for artificial oxygen carrying solutions**

| Application | Context |
|---|---|
| 'Bridge oxygenator' | Avoid, reduce or delay blood transfusion |
| Local tissue ischaemia | Percutaneous transluminal coronary angioplasty |
| | Necrotizing enterocolitis (intraluminal in gastrointestinal tract) |
| Gas absorber (emulsified PFCs only) | Cardiopulmonary bypass |
| | Decompression sickness |
| Reduction of tumour hypoxia | Increases effectiveness radiotherapy |
| (Partial) liquid ventilation (pure PFCs only) | Acute respiratory distress syndrome |
| Organ preservation | Transplantation medicine |
| Cell culture medium | |
| Nitric oxide scavenging | Possible benefit of HBOCs in sepsis |
| Radiology (PFCs only) | |

HBOC = haemoglobin-based oxygen carrier; PFC = perfluorocarbon

**TABLE 2.**
**Characteristics of the ideal artificial oxygen carrier**

| |
|---|
| Sufficient oxygen uptake and delivery at physiological oxygen tension |
| No significant toxic or physiological effects |
| No disease transmission |
| No antigenic properties |
| Sufficient intravascular half-life |
| Long shelf life |
| Easy to use and store |
| Universally compatible |
| Readily available in large quantities |
| Low cost |

## HAEMOGLOBIN-BASED OXYGEN CARRIERS

Hb is the oxygen-carrying protein of red blood cells. In the red blood cell it exists as a tetramer composed of two alpha and two beta globin chains ($\alpha_2\beta_2$). Each chain holds an iron-containing porphyrin ring and each iron atom can bind reversibly with one oxygen molecule. HBOC solutions can be prepared by releasing Hb from bovine or time-expired human erythrocytes, or less commonly biosynthesized using recombinant techniques.

Free Hb solutions were administered intravenously as long ago as the late 19th century. These early solutions had a number of toxic effects including vasopressor action, activation of complement and coagulation pathways, nephrotoxicity, antigenic effects, histamine release and deposition of iron. It became clear that toxicity was primarily associated with stromal remnants. These resulted from early methods of releasing Hb from erythrocytes that involved 'bursting' red cells with limited subsequent purification. By the 1970s, new purification techniques allowed the production of 'stroma free' Hb (containing less than 1–2% stromal elements) that resolved many of the early problems. However, unmodified stroma-free Hb solutions were still associated with a number of problems that limited their use as AOCs:

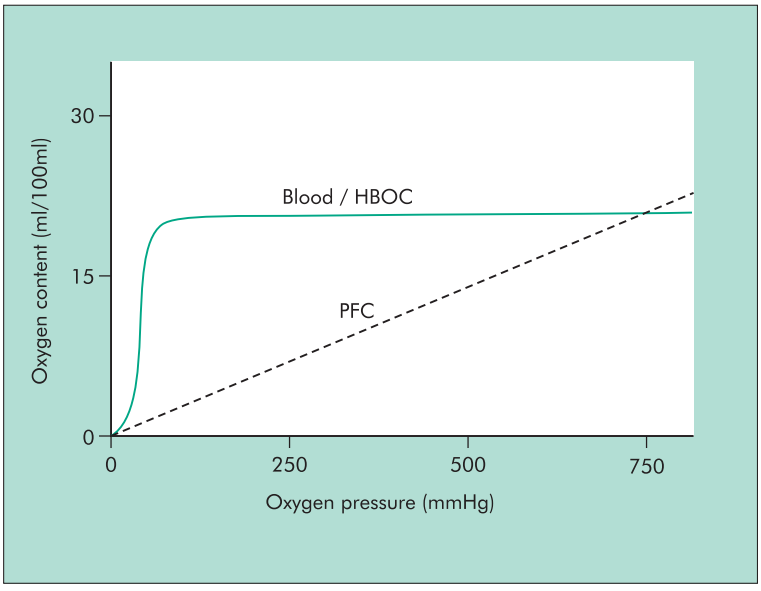
- Absence of 2,3-diphosphoglycerate (2,3-DPG) and higher pH outside the red cell causes a left shift in the oxygen Hb dissociation curve (*Figure 1*). The $P_{50}$ of free Hb (the partial pressure of oxygen which results in 50% oxygen saturation of Hb) is therefore lower and its avidity for oxygen higher. Oxygen uptake in the pulmonary circulation is facilitated but its release to the tissues is reduced.
- Free Hb is unstable in solution and quickly dissociates into $\alpha\beta$ dimers that are rapidly filtered and excreted in the urine resulting in a short intravascular half-life (1–2 hours) and renal toxicity.
- The ferric group of iron in free Hb is an avid scavenger of nitric oxide (NO) resulting in an increase in both systemic and pulmonary pressures.
- Oxygen dissociating from free Hb can uncouple an electron, creating methaemoglobin and a superoxide radical. This causes oxidative stress that persists because of the absence of methaemoglobin reductase and superoxide dismutase. HBOCs are therefore easily oxidized to methaemoglobin and must be stored anaerobically.
- Free Hb particles are osmotically active and unmodified solutions are hyperosmolar.

Cross-linking, conjugation or microencapsulation of extracted Hb, or genetic engineering, can overcome many of these problems (*Table 3*).

Human-derived HBOCs, produced by lysis of erythrocytes obtained from outdated banked blood, have a $P_{50}$ reduced from 27 mmHg to 12–13 mmHg. Hb cross-linkage, pyridoxylation or carboxymethylation of the terminal Hb residues can reduce this high oxygen affinity. However, human-derived HBOC solutions have the disadvantage that they are produced from the limited available stores of donated blood.

As an alternative animal-derived Hb (predominantly bovine) can be used with similar methods of Hb modification to those used for human-derived HBOCs. Bovine Hb has a number of advantages over human Hb in the manufacture of HBOCs. It combines with chloride rather than 2,3-DPG and therefore has an extraerythrocytic $P_{50}$ of around 28–30 mmHg. It thus releases oxygen more readily to the tissues. It can also be obtained at relatively low cost from an abundant supply and this makes it an attractive proposition commercially. Concerns about the potential risk of new variant Creutzfeldt–Jakob disease contamination have now been alleviated. Herds from which Hb is derived are well characterized and managed, and multiple step purification processes are used. HBOCs using Hb derived from other species, including shark and crocodile, are currently under investigation.

***Figure 1. Comparison of oxygen content between blood, haemoglobin-based oxygen carriers (HBOCs) and perfluorocarbons (PFCs).***



Prolonging intravascular retention and reduction of colloid osmotic pressure can be achieved by a number of processes. Colloid osmotic pressure is proportional to particle number rather than size. Increasing particle size by polymerization of Hb increases the number of oxygen binding sites relative to the osmotic pressure. Polymerization of Hb molecules by glutaraldehyde or raffinose results in the formation of stable tetramers and of tetramer polymers. A purification process is then required to remove non-reacted Hb, single tetramers and higher order polymers. Polymerization, however, reduces oxygen binding cooperativity.

Alternatively, cross-linking of the α-substitutes (e.g. diaspirin cross-linked Hb; DCLHb) can be used to reduce tetramer dissociation. Hb can also be conjugated to macromolecules (e.g. dextran or polyethylene glycol; PEG) to produce solutions that acquire some of the properties of the molecules to which the Hb is conjugated (e.g. PEG-Hb). Using these methods, renal filtration of Hb moieties and renal toxicity is reduced and some cooperativity of oxygen binding can be maintained.

More recently encapsulation of Hb molecules to produce pseudo-erythrocytes has been used to prolong intravascular retention. Liposomal encapsulation of Hb has been achieved and the concept has been further developed with the production of smaller nanocapsules using polyglycone or polylactone membranes. Encapsulation allows inclusion of erythrocytic molecules such as 2,3-DPG and methaemoglobin reductase within the pseudoerythrocyte to overcome problems associated with free Hb.

An alternative to these extraction and modification processes is the synthesis of Hb molecules using recombinant techniques (e.g. rHb 1.1, Somatogen, Boulder, CO). Modern biogenetic techniques allow the possibility of producing 'designer' Hbs with specific amino acid sequences dictating tertiary and quaternary molecular structure and therefore function. Modifications can be made to the globin chains expressed leading to a reduction in oxygen affinity and maintenance of the tetramic configuration after infusion. A second-generation recombinant Hb has been synthesized with the receptor site for NO blocked, potentially reducing vasopressor effects.

## CLINICAL EXPERIENCE

A number of HBOCs have completed safety studies, and phase III studies to test efficacy are underway. The US Food and Drug Administration (FDA) have specified that for any potential AOC it will only consider applications for regional perfusion, e.g. percutaneous transluminal coronary angioplasty (PTCA), acute haemorrhagic shock and perisurgical applications. The FDA have stated that it will accept avoidance of allogeneic blood transfusion as an end point but not secondary markers of improved tissue oxygenation. It is in this direction that phase III trials are currently aimed.

Baxter (Deerfield, IL) has ceased the production of its α-α cross-linked haemoglobin tetramer, DCLHb solution marketed as HemAssist™. Two major multicentre phase III trials were discontinued after reports of excess mortality in patients receiving DCLHb in one of the trials. Two reports have been published on European and American trials with this product (Saxena et al, 1999; Sloan et al, 1999) confirming a worse outcome in patients receiving DCLHb. Although no specific reason has been cited, it is likely that a number of factors played a part. The study groups were heterogeneous with a high mortality of their own. It has been suggested that the vasopressor effect of the HBOC solution resulted in masking of shock thus causing less fluid to be given during resuscitation, and so reducing tissue perfusion.

**TABLE 3.**
**Haemoglobin-based oxygen carrier solutions**

| Product name | Developer | Haemoglobin source | Modification |
|---|---|---|---|
| HemAssist™ (DCLHb) | Baxter Healthcare | Human | Diaspirin cross-linked |
| PolyHeme™ (Poly-SFH-P) | Northfield Labs | Human | Pyridoxylated, glutaraldehyde polymerized |
| Hemopure™ (HBOC-201) | Biopure Corporation | Bovine | Glutaraldehyde polymerized |
| Hemolink™ | Hemosol | Human | O-raffinose cross-linked |
| PHP conjugate | Apex Bioscience | Human | Pyridoxylated, polyethylene glycol conjugated |
| Polyethylene glycol-haemoglobin | Enzon | Bovine | Polyethylene glycol conjugated |
| Optro™ RHb 1.1 | Somatogen | Recombinant human | *Escherichia coli* expressed. Fused α dimers |

DCLHb = diaspirin cross-linked haemoglobin; HBOC = haemoglobin-based oxygen carrying solution; PHP = pyridoxylated haemoglobin polyoxyethylene; Poly-SFH-P = polymerized pyridoxylated stroma free haemoglobin; RHb = recombinant haemoglobin

Baxter is now working with Somatogen to develop a second-generation recombinant Hb in which the NO receptor site has been blocked. Results from animal experiments suggest that vasoconstriction does not occur when this product is infused.

Hemosol (Mississauga, Ontario, Canada) have completed a Canadian phase III trial in patients undergoing cardiac surgery demonstrating that HemoLink™ can be used safely to reduce blood transfusion requirements but these data are as yet unpublished in a peer-reviewed form. Further trials are taking place in coronary artery bypass surgery in the USA, UK and Canada. Results of these trials are expected to be available during 2003.

Northfield (Evanston, IL) have reported results on a human polymerized Hb product (PolyHeme™) demonstrating reduced allogenic blood transfused in trauma and surgery patients with acute blood loss (Gould et al, 1998). Phase III trials are now underway using higher doses to ensure lack of adverse effects.

Biopure's (Cambridge, MA) phase III trials are underway with Hemopure™ in surgical patients for intraoperative blood loss. Hemopure™ is already licenced for human use in South Africa. A similar bovine Hb product by the same company is currently approved for veterinary use in anaemic dogs in the USA.

Enzon's (Bridgewater, NJ) phase II trials with PEG-Hb are focussing on its enhancement of radiosensitivity of tumours in patients with cancer and Apex Bioscience (Curacyte Health Sciences, Munich, Germany) are using their pyridoxylated haemoglobin polyoxyethylene conjugate for NO scavenging in patients with septic shock.

## PERFLUOROCARBONS

PFCs are a group of novel synthetic compounds, first developed over 30 years ago, with unique physical and chemical properties (*Table 4*). They are low molecular weight (400–500 Dalton) molecules. The electron-rich fluorine atoms that surround the carbon backbone repel each other resulting in weak intermolecular interaction, which facilitates gas molecule insertion and carriage. Although there are countless possible PFCs, only a few are suitable for clinical use.

Oxygen is soluble in PFCs and oxygen carriage is directly proportional to both the partial pressure of oxygen in the blood (Henry's law) and to the concentration of PFC in the blood. In order to maximize oxygen carriage and delivery during PFC therapy the inspired oxygen concentration is normally increased (*Figure 1*). Oxygen is unbound and is therefore readily available to the tissues. Nearly all oxygen is extracted from PFCs before any is released from Hb.

PFCs must be emulsified before they can be used intravenously as bridge oxygenators. Unemulsified PFCs have been used for liquid ventilation (although benefit has only been shown in paediatric practice) in patients with severe acute lung injury or acute respiratory distress syndrome resistant to other therapies. Irrespective of route of administration, PFCs are not significantly metabolized within the body and are expired by the lungs. However, they are known to cycle through the reticuloendothelial system. Median emulsion droplet sizes can be as small as 0.2 μm, far smaller than red cells, allowing carriage of oxygen in small vessels and potentially more efficient tissue oxygenation via the microvasculature. Early PFCs were emulsified in agents associated with a significant incidence of adverse drug reactions. Newer formulations seem much safer.

The first generation of PFC to be used clinically was Fluosol DA, developed in 1976 and introduced by the Green Cross Corporation (Osaka, Japan). Owing to its viscosity it was limited to a 20% solution. It was unstable and was stored frozen and then mixed before use. Early trials showed limited benefits and a number of adverse effects became apparent including hepatosplenomegaly (because of reticuloendothelial system retention), anaphylactoid reactions and complement activation, thought to be related to the pluronic emulsifier. However, the product did gain FDA approval in 1989 for use for distal perfusion during PTCA until that indication was rendered obsolete by the introduction of autoperfusion angioplasty catheters.

**TABLE 4.**
**Properties of perfluorocarbons**

| |
|---|
| Chemically inert |
| Colourless |
| Odourless |
| High gas solubility (carbon dioxide>oxygen>nitrogen) |
| High density |
| Low viscosity |
| Low surface tension |
| Radio-opacity |
| High compressibility |
| Immiscible in aqueous systems (requires emulsification) |

Production of Fluosol-DA ceased in 1994, but it remains the only oxygen carrier to have achieved FDA approval for clinical use.

A second generation of emulsified PFCs has now been developed and these products are currently undergoing clinical trials. The use of egg yolk lecithin as an emulsifier has removed the problems of complement activation and solutions are now more stable. Some adverse effects still remain, including flu-like symptoms and a transient fall in platelet count, although bleeding time and clotting profile remain unaffected. The first product is Oxygent™ (Alliance Pharmaceutical Corporation, San Diego), which is the emulsified version of Perflubron (LiquiVent), the only agent currently with regulatory approval for use in liquid ventilation. Oxygent has now been evaluated in five phase II trials, where it has been used as part of an 'augmented normovolaemic haemodilution' regimen and in patients undergoing cardiac bypass.

In one study of 147 orthopaedic patients, Oxygent was more effective than autologous blood or colloid solution in reversing physiological transfusion triggers (Spahn et al, 1999). Oxygent has now completed a phase III trial in Europe for use in non-cardiac surgery patients, showing a reduction in donor blood requirements and an increase in avoidance of blood transfusion in the subgroup of patients receiving Oxygent with blood loss >20 ml/kg (those specifically targeted) (Spahn et al, 2001). A recent phase III trial in cardiac surgical patients has been terminated early because of an excess of adverse neurological outcomes in the treatment group. A new European and Canadian phase III trial in non-cardiac surgery is due to start in the near future.

## KEY POINTS

- The availability of blood for transfusion is decreasing while demand continues to increase.
- Haemoglobin-based oxygen carriers (HBOCs) and perfluorocarbons (PFCs) are being developed as artificial oxygen carriers that can safely be administered intravenously.
- HBOCs are derived by modification of extra-erythrocytic bovine or human haemoglobin or can be biosynthesized de novo.
- PFCs are novel synthetic low molecular weight fluorinated hydrocarbons that dissolve oxygen and can be intravenously administered in emulsified form.
- Clinical trial data on HBOCs and PFCs are limited at present and further work will be required before these exciting products enter clinical practice.

The second PFC emulsion is Oxyfluor™ (HemaGen, Baxter). Its ability to absorb gaseous microemboli in patients undergoing cardiopulmonary bypass surgery is currently undergoing evaluation in stage II studies. No peer reviewed published data are yet available.

## THE FUTURE

AOCs have great theoretical potential but there is as yet limited clinical data. Initial areas of use are likely to include emergency medicine, planned major surgery and remote environment use (e.g. military) where blood transfusion is impractical. The cost-benefit comparison of AOCs with allogeneic blood transfusion remains unclear. It is likely that a number of products will be approved for clinical use in the near future, but more studies are needed to demonstrate clinical utility and to more clearly define the specific indications for these exciting new products. HM

*Conflict of interest: none.*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**Further reading**


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