Haemoglobin-based oxygen carriers: indications and future applications

This article describes current oxygen-carrying solutions, four new products and new indications to increase the benefit/risk ratio of haemoglobin-based oxygen carriers compared to blood. Indications include when blood is not available, if blood is contaminated, is refused or contraindicated, and for organ preservation.

During the 1970s and 1980s there were concerns over the safety of blood (Busch, 2001; Van Hemelrijck et al, 2014) and excessive use of blood transfusions (Hébert et al, 1998). This situation heralded a considerable investment in production and clinical testing of haemoglobin-based oxygen carriers. Since 2000, the specialty of transfusion medicine has evolved – improving the safety of red blood cell transfusion and implementing guidelines for blood product administration, so that transfusion is relatively safe, mostly readily available and generally acceptable. In severe acute anaemia, when blood is refused or not available, haemoglobin-based oxygen carriers may be used to increase oxygen delivery and reverse tissue ischaemia. Blood may not be available because the donor pool for blood is shrinking (Zou et al, 2008). Although 75 million units are collected worldwide, 13 million are not tested for human immunodeficiency virus (HIV) (Zou et al, 2008). Haemoglobin-based oxygen carriers, lacking disease transmission and need for cross-matching or refrigeration, are an alternative in the developing world to allow more time for scaling up of blood transfusion banks and services.

The primary reasons for using haemoglobin-based oxygen carriers are when:

1. Blood supplies cannot be cross-matched
2. Blood is contaminated or transfusion may produce adverse events
3. Blood is unavailable or supplies are limited
4. Blood transfusion is refused
5. Avoidance of frequent blood transfusions for chronic diseases such as sickle cell with acute anaemia is preferred to decrease the consequences of repeated transfusion (i.e. transfusion reactions, alloimmunization)
6. Preservation and resuscitation of organ transplant tissue is indicated before transplantation.

Haemoglobin-based oxygen carriers are acellular with rheological advantages over blood. Blood is a non-Newtonian fluid, so haemoglobin-based oxygen carriers may better maintain microvascular blood flow and tissue oxygenation. Because the one currently available haemoglobin-based oxygen carrier product has a 3-year shelf life without refrigeration, the potential situations in which current and the next generation haemoglobin-based oxygen carrier products could be indicated are extensive (*Table 1*).

This review identifies new indications for haemoglobin-based oxygen carriers in developing countries when blood is not available, highlights the differences between four new emerging haemoglobin-based oxygen carriers from those previously tested and describes current ongoing investigations of Hemopure (HBOC-201) – the only product approved for human use in expanded access for patients with life-threatening anaemia, for pre-hospital resuscitation and transplant organ preservation.

New and existing haemoglobin-based oxygen carriers

There have been articles (Alayash, 2014; Lewis and Ross, 2014; Van Hemelrijck et al, 2014) and a 750-page book

Table 1. Indications for haemoglobin-based oxygen carriers

Pre-hospital administration, in the field and during en route care
Administration in remote environments for disaster response or in combat environments
Blood transfusion refusal, e.g. Jehovah's Witness
Decrease the use of red blood cells when low volume transfusions might be indicated
Red blood cells are indicated but suitable resources are limited, i.e. limited supply or resource for blood type, high volume transfusion requirement with inadequate supply
Improvement in oxygen-carrying capacity for priming of cardiopulmonary bypass, coronary revascularization as a result of low rheology
Blood is contraindicated, e.g. auto-immune haemolytic anaemia, cross-matching impossible
Repeated blood transfusion can have adverse events, e.g. sickle cell disease leading to haemosiderosis
For transplant organ preservation and resuscitation

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(Kim and Greenburg, 2013) on existing and new haemoglobin-based oxygen carriers, demonstrating that there is no lack of interest in finding an alternative to blood that can support tissue oxygen delivery.

The clinical use of existing haemoglobin-based oxygen carriers has been limited as a result of adverse effects reported from clinical trials and a meta-analysis indicating an increased occurrence of myocardial ischaemia and death (Natanson et al, 2008), although the conclusions and methodology of this were criticized (see below). The adverse effects reported in clinical trials include cardiovascular, pulmonary, gastrointestinal, renal and haematological (Silverman and Weiskopf, 2009). Hypertension has been attributed to endothelial nitric oxide scavenging and to haem-mediated oxidative side reactions and over-supply of oxygen (Alayash, 2014). As a result new generations of haemoglobin-based oxygen carriers to minimize nitric oxide binding and haem-mediated side reactions are emerging beyond the existing use of Hemopure HBOC-201 (HbO2 Therapeutics, Souderton, PA, United States), the only haemoglobin-based oxygen carrier approved for human use (in South Africa and Russia, but not US or Europe).

New haemoglobin-based oxygen carriers

Given the adverse events reported with the use of haemoglobin-based oxygen carriers, efforts continue to improve their safety. Recent studies have evaluated both the addition of bioactive substances to haemoglobin-based oxygen carrier solutions and the formation of nitric oxide-containing molecules in vivo to alleviate the vasoconstriction and increased blood pressure seen on administration of haemoglobin-based oxygen carriers. Four new haemoglobin-based oxygen carriers with additional bioactive particles to mitigate side effects are under investigation (Lewis and Ross, 2014):

1. Polynitroxylated pegylated haemoglobin (PNPH), a peg-haemoglobin with nitroxide moieties (Synzyme Technologiea LLC, Irvine, California, United States)
2. OxyVita (OxyVita Inc. New Windsor, New York, United States), a zero-linked polymerized bovine haemoglobin
3. Hemoxycarrier (Hemarina, Morliax, France), an acellular haemoglobin found in the marine worm *Arenicola marina*
4. MP4CO, a carboxylated, pegylated bovine haemoglobin (Sangart Inc., San Diego, California, United States) (*Table 2*).

Pegylation of haemoglobin reduces interaction of haem proteins with the vascular endothelium and decreases nitric oxide scavenging compared with cross-linked or native haemoglobin. The nitroxide moieties on PNPH have superoxide dismutase-mimetic activity, which reduces oxidative stress and decreases the free radical-mediated cytotoxicity of cell-free haemoglobin. Re-engineering of haemoglobin into an oxidatively stable molecule with a stabilizing protein such as haptoglobin or haemopexin provides protection against oxidative damage to haemoglobin subunits and surrounding proteins during erythropoeisis (Alayash, 2014).

Carbon monoxide is a mediator of vascular tone and cellular signalling with vasodilatory and anti-inflammatory properties, which may make carboxylation a beneficial modification to haemoglobin-based oxygen carriers. In a transgenic mouse model of sickle cell disease MP4CO reduced microvascular stasis after hypoxia or reperfusion compared with MP4Ox and saline (Belcher et al, 2013). OxyVita and PNPH are also produced in carboxylated formulations (Lewis and Ross, 2014). Variations of stimulatory molecules of soluble guanylyl cyclase (pyrazolopyridinylpyrimidines), termed 'BAY' stimulators, have been tested in animal studies and their concomitant use with haemoglobin-based oxygen carriers (Raat et al, 2013) proved effective in reducing haemoglobin-based oxygen carrier-induced vasoconstriction through direct stimulation of soluble guanylyl cyclase with bypass of nitric oxide in vascular smooth muscle cells.

Table 2. Characteristics of four new haemoglobin-based oxygen carrying solutions

Agent	Company	Description	pH	P_{50} O_2	Colloid oncotic pressure	Size/weight	Half-life
Polynitroxylated pegylated haemoglobin [C]	SynZyme (Irvine, CA)	PEG and nitroxide moieties → superoxide dismutase mimetic activity. Also prepared as carbon monoxide formulation	7–8	10 mmHg	45 mmHg	120 kD	20 h
OxyVita [C]	OxyVita (New Windsor, NY)	Very large haemoglobin polymers → decreased extravascular extravasation. Powder form and carbon monoxide formulation available	7.4	6 mmHg	3 mmHg	17 MD	6 h
Hemoxycarrier	Hemarina (Morlaix, France)	Natural extracellular haemoglobin. Large molecule with inherent superoxide dismutase mimetic activity	7–8	7.05 mmHg	1 mmHg	3600 kD	50 h
MP4CO	Sangart (San Diego, CA)	PEG haemoglobin with carbon monoxide	7.4	6 mmHg	55 mmHg	95 kD	20 h

[C] indicates that carboxylated formulation is also produced. P_{50} O_2 = partial pressure of oxygen of haemoglobin at 50% saturation; PEG = polyethylene glycol. From Lewis and Ross (2014)

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The formation of S-nitrosohaemoglobin has been extensively studied by researchers as a means of delivering nitric oxide to hypoxic tissues in vivo. S-nitrosohaemoglobin formation occurs under natural conditions in vivo and can ultimately lead to the formation of sub-unit nitric oxide reductases, which could increase blood flow to oxygen-deprived tissues, much like free nitric oxide (Galvagno and Mackenzie, 2013). Interest in alternative vasodilatory mechanisms continues to advance and may serve as a means to manipulate the aberrant effects of haemoglobin-based oxygen carriers on the vasculature.

Previously tested haemoglobin-based oxygen carriers and reason for discontinuation

MP4 (Sangart Inc., San Diego, CA) was designed with low partial pressure at 50% saturation (P50 = high affinity of haemoglobin to oxygen = 5–6 mmHg), low haemoglobin concentration (haemoglobin 4.2 g/dl) and high oncotic pressure (49 mmHg) to minimize nitric oxide binding, extravasation and preserve functional capillary density in anaemia and shock states. MP4 was tested in elective surgery, chronic critical limb ischaemia and prevention of hypotension. There was an increased incidence of bradyarrhythmias, blood pressure elevation, nausea, liver and pancreatic enzyme elevation, so Sangart stopped production.

PolyHeme (Northfield Laboratories, Evanston, Il) has been tested in situations when blood is not available at the scene of an injury, during transport to definitive care, and in the hospital instead of transfusion because of religious objection, blood incompatibility or shortage. Serious adverse events reported from trials include pneumonia, respiratory failure, multi-organ failure, haemorrhagic shock, hypercoagulable state, coagulopathy and myocardial infarction. Thirty-day mortality (13.4% Polyheme and 9.6% control) in their pre-hospital trauma trial and 30-day mortality for injury sub-groups were significantly higher in those randomized to Polyheme (Moore et al, 2009), leading to the failure of regulatory approval for PolyHeme and resulting in closure of Northfield, the manufacturer.

PEG-Hb (Enzon Pharmaceuticals, Piscataway, NJ) was developed with an indication to increase tumour oxygenation, in order to enhance radiosensitivity of susceptible tumours. Side effects included mild hypertension, dysphagia, nausea and vomiting, and manufacture was discontinued.

Hemolink (Hemosol Inc, Mississauga, Ontario, Canada) production was discontinued as a result of hypertension, myocardial infarction and gastrointestinal side effects observed in clinical trials.

Somatogen rHb1.1 (Baxter Healthcare Corp, Deerfield, Il) was used in the treatment of traumatic haemorrhagic shock with prehospital administration in lieu of blood and in hospital as an adjunct to standard care including red blood cells. Both trials were stopped because of high mortality. Pancreatitis was a notable adverse effect. rHb2.0 was developed to replace rHb1.1 and to reduce the incidence of vasoactivity and gastrointestinal effects. Complement activation was a notable side effect. All the companies producing the haemoglobin-based oxygen carriers listed above have either stopped their manufacture or gone bankrupt.

Currently available haemoglobin-based oxygen carrier

One product, Hemopure (HBOC-201), has been made available for compassionate clinical use in the South Africa, USA, Australia, and Europe, including the UK. Hemopure is a gluteraldehyde polymerized solution of bovine haemoglobin in modified lactated Ringer's solution, with a haemoglobin concentration of 13 g/dl and a colloid oncotic pressure of 25 mmHg. Hemopure does not require reconstitution and compares favourably to whole blood in many of its physiological and oxygen-carrying properties. Hemopure can be stored at 2–30°C without deterioration over a 3-year shelf life, and does not transmit disease.

Safety and efficacy of Hemopure

Hemopure has been administered to over 800 humans, and has undergone 22 clinical trials, more than any other haemoglobin-based oxygen carrier. In HEM-0115, a phase 3 prospective randomized clinical trial, perioperative infusion of Hemopure (n=350) was compared with packed red blood cells (n=338) transfusions (haemoglobin ~25 g/dl) in patients undergoing elective orthopaedic surgery. Transfusion avoidance was demonstrated. In the intent to treat analysis mortality was not different (2.9% *vs* 1.8% respectively, P=0.3). However, in older patients (>80 years) Hemopure's safety profile was inferior to packed red blood cells (7.7% excess incidence of serious adverse events; 25.1% *vs* 17.5% respectively, P=0.02). The risk of serious adverse events (0.14/patient) and_mortality was identical in those who received up to 10 units of Hemopure and those who received three units of packed red blood cells (Jahr et al, 2008).

There was 96% transfusion avoidance for 24 hours, providing adequate time for blood banks to marshal additional blood for transfusion. Within 1 week there was a 76% transfusion avoidance in those randomized to Hemopure, making this a practical alternative when blood is unlikely ever to become available (Galvagno and Mackenzie, 2013). Elevations of blood pressure (averaged 23 mmHg on first administration of Hemopure and 11 mmHg on subsequent administration) were transient. Only one of 350 patients had a serious adverse event of intractable elevated blood pressure, and other blood pressure changes resolved or were treated adequately with pharmacological intervention, indicating that this is a transient rise in blood pressure, not chronic hypertension.

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HEM-0114 trial findings

In a prospective randomized multicentre controlled clinical trial 150 non-cardiac surgery patients were randomized to receive either blood (n=77) or HBOC-201 (n=83) up to 7 units after which cross-over to blood was required. The efficacy end-point was elimination or reduction of transfusion during 28 days, and significantly less blood use (3.2 units *vs* 4.4 units, P=0.004) occurred in those randomized to haemoglobin-based oxygen carrier and 43% avoided blood transfusion.

No differences were found in serious adverse events (P=0.73), 30-day mortality (P=1.0), time to intensive care unit (P=0.15) or hospital discharge (P=0.53). Haemoglobin-based oxygen carrier was associated with a greater incidence of non-serious adverse events (P<0.001) such as mild hypertension and fever. Transient jaundice, associated with higher doses of HBOC-201, was reported in 31 (37.3%) HBOC-201 subjects and in one (1.3%) subject given red blood cells (P<0.001). All treatment-associated instances of jaundice in the HBOC-201 group were resolved, and approximately 50% of these had a duration of 3 days or less. There were no reports of liver failure with treatment-associated jaundice. The skin pigmentation observed in most cases was a pre-hepatic jaundice based on increased bilirubin load and was consistent with physiological processing of HBOC-201 to bilirubin (Van Hemelrijck et al, 2014).

This HEM-0114 trial was conducted in 1998–9, but the results were written up and interpreted in 2014 with current knowledge of haemoglobin-based oxygen carrier and transfusion medicine. The absence of cardiac adverse events and safety signals (except for blood pressure elevations) is consistent with results from other HBOC-201 clinical trials which reported the absence of cardiotoxicity. The results of the HEM-0115 and HEM-0114 trials lend support for continued clinical development for indications where blood transfusions are not an option. Recently published commentary further discusses situations where the use of haemoglobin-based oxygen carriers could be beneficial (Lewis and Ross, 2014).

Additional uses of Hemopure (HBOC-201) in developing countries

In developing countries where blood transfusion services are inadequate, haemoglobin-based oxygen carriers could be useful as an oxygen-carrying alternative, since haemoglobin-based oxygen carrier infusion requires fewer resources (no refrigeration, long shelf-life), less infrastructure (no cross-match or screening), less personnel training and costs than are required for collection, testing and administration of blood. Transfusion complications, their cost and haemovigilance programmes alone consume more than the per-patient budgets of most developing countries (Shander et al, 2007).

Hemopure does not transmit diseases and therefore has important medical and economic advantages over blood for avoidance of transfusion-related HIV transmission and in Middle Eastern, Mediterranean and African countries. The use of Hemopure may avoid transfusion of blood contaminated with human herpes virus 8 (Kaposi's sarcoma) which is associated with increased mortality (Hladik et al, 2012). Maternal deaths from post-partum hemorrhage are a major issue in developing countries and mortality might be avoided with haemoglobin-based oxygen carriers stockpiled during pregnancy, to use until blood can be obtained and surgical intervention initiated. Benefits of haemoglobin-based oxygen carriers over blood are summarized in *Table 3*.

Table 3. Benefits of haemoglobin-based oxygen carriers over blood

Ready to use with no planning, no equipment, blood collection or blood processing laboratories and associated costs. HBOC-201 has no known disease transmission
No donor recruitment, no collection (personnel/equipment), no blood transport and distribution
No pre-transfusion preparation, limited blood haemovigilance and paperwork
No processing and separation of components
No waste as HBOC-201 has 30–50 times longer shelf-life than blood until expiration date, allowing stockpiling for battlefield readiness, disasters, managing post-partum haemorrhage in remote areas, in emergency medical ambulances and helicopters
HBOC-201 needs no refrigeration, hence potential availability in two-thirds of the developing world with inadequate blood bank facilities
To avoid disease transmission (HIV and human herpes virus 8)
Universally compatible, could replace all un-cross-matched blood use. No transfusion errors as no cross-match
Immediately off-loads oxygen, no 2,3-diphosphoglycerate-dependent tissue oxygen off-loading
May be used by Jehovah's Witnesses
Provides oxygenation when blood not an option, e.g. auto-immune haemolytic anaemia
Extends the useful life of donor organs awaiting transplant

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Ongoing testing of Hemopure (HBOC-201)

Animal studies have shown that tissue oxygen delivery with various concentrations of Hemopure provided higher functional capillary density and was superior to blood when the lowest dose of Hemopure given (4 g/dl) balanced oxygen delivery and vasoconstriction, suggesting that in humans there may be benefit in lowering Hemopure haemoglobin concentrations (Ortiz et al, 2014).

An ongoing clinical trial of Hemopure in America (ClinicalTrials.gov Identifier:NCT01881503) is evaluating expanded access for patients with life-threatening anaemia for whom blood transfusion is not an option, and a pre-hospital randomized controlled (normal saline control) trauma study of Hemopure in subjects with haemorrhagic shock and prolonged transport times is about to begin in Melbourne (The Alfred and Royal Melbourne Hospitals) and Ambulance Victoria, Australia.

Meta-analysis and National Institutes of Health/Food and Drug Administration meeting

A meta-analysis published in 2008 (Natanson et al, 2008) to coincide with the National Institutes of Health/Food and Drug Administration meeting suggested that there was a 'class effect' of haemoglobin-based oxygen carriers increasing mortality and myocardial infarction and, together with the meeting discussions, recommended that all human testing cease (Silverman and Weiskopf, 2009). This meta-analysis was criticized for its methodology, omission of data, conclusions and content in six letters to the Editor published in September 2008 (*JAMA* 300: 1296–9).

Instead of separating the 22 clinical trials of Hemopure (39% of the total data) which had different controls, including crystalloids, packed red blood cells and colloids, the meta-analysis put them all together as one trial. It also included five different haemoglobin-based oxygen carriers with different comparators which is unacceptable meta-analysis methodology. Hemassist was included among five haemoglobin-based oxygen carriers, despite having been removed from the market more than 10 years ago. Removal of Hemassist from the meta-analysis reversed the findings of increased myocardial infarction and mortality. Hemopure specifically reverses myocardial ischaemia (Alayash, 2014) and in modelling is not a significant factor causing serious adverse events, so a 'class effect' for haemoglobin-based oxygen carriers seems unlikely. Of the total data included in the meta-analysis 63% originated from non-peer reviewed sources (newspapers and third party presentations). Little was known about study design, controls and dose. The aggregation included safety studies (in contradiction to the stated meta-analysis exclusion criteria), different randomization schemes (1 to 1 and 2 to 1), patient types, and trials with very different design and controls (blood, crystalloid, colloid).

Haemoglobin-based oxygen carriers for organ preservation before transplantation

Other innovative research focuses on alternative uses for haemoglobin-based oxygen carriers for preservation of organ function. Because the demand for viable transplantable organs outweighs the supply, interest has peaked in haemoglobin-based oxygen carrier use for organ preservation and extracorporeal resuscitation. Ischaemia–reperfusion injuries are inherent to harvesting and transplantation of organs. The standard preservation techniques include hypothermic cold storage in a preservation fluid with electrolytes, amino acids and other molecules designed to maintain cell integrity. Noticeably absent from the solutions is oxygen. Cells, therefore, experience depletion of adenosine triphosphate and a build up of toxic substances during cold storage which can lead to decreased organ viability (Hosgood et al, 2014).

Adding haemoglobin-based oxygen carriers to cold storage solutions is of interest to maintain organ viability. One study discovered a cardioprotective effect of polymerized human placenta haemoglobin (PolyPHb) after perfusing a rat heart with the haemoglobin-based oxygen carrier, storing it at a cool temperature for 9–14 hours, and then replacing blood perfusion. The haemoglobin-based oxygen carrier-perfused heart had lower inflammatory mediator activity than the heart perfused with storage solution alone, which researchers attributed to reduced reactive oxygen species production, mitochondrial stabilization and apoptotic inhibition provided by PolyPHb (Wei et al, 2011). PolyPHb was also shown to protect kidneys from ischaemia reperfusion injuries upon pretreatment which could have further implications for organ preservation (Li et al, 2013).

Other haemoglobin preparations have been studied as additives to cold storage solutions including polymerized porcine haemoglobin and an extracellular haemoglobin of the marine invertebrate *Arenicola marina* (Hemarina-M101). Polymerized porcine haemoglobin was studied as an additive to storage solutions during small bowel transplantations in rats. It was found to elevate adenosine triphosphate concentrations, increase tissue respiration, stabilize pH, and decrease lactate production in tissue compared to standard solutions (Huang et al, 2014). French investigators demonstrated increased metabolic activity of cold-stored kidney epithelial cells when Hemarina-M101 was added to six different standard solutions and compared to the standard solutions alone. Hemarina-M101 also reduced lactate dehydrogenase production significantly in four of the solutions and improved kidney function and histological integrity following reperfusion compared to the most common standard solutions (Thuillier et al, 2011).

Conclusions

Clearly, the future of haemoglobin-based oxygen carriers is not limited to trauma settings as ischaemia–reperfusion protection and organ preservation are areas of increasing

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interest for the scientific community. Using haemoglobin-based oxygen carriers to pretreat organs, prevent inflammatory mediator production or to enhance cold standard solutions is a new frontier for haemoglobin-based oxygen carrier research. **BJHM**

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KEY POINTS

- Worldwide interest remains in finding an alternative to blood transfusion for oxygen carriage.
- One currently available product Hemopure – HBOC-201 – remains to be approved by American regulatory authorities, but is still under study for 'compassionate use' when blood is refused, and in pre-hospital care of haemorrhaging trauma patients when blood is not available.
- Four new haemoglobin-based oxygen carriers could show improved benefit/risk ratio.
- Consideration should be given to haemoglobin-based oxygen carrier use in developing countries, until safe blood transfusion banks and services can be scaled up and blood supplies secured.
- The medical and economic benefits of haemoglobin-based oxygen carriers in such countries may outweigh the risks.
- Haemoglobin-based oxygen carriers appear superior to blood for extra-corporeal organ resuscitation and preservation before transplantation.

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