Haemoglobin-based oxygen carriers: is the benefit worth the risk?

When blood is indicated but not available, haemoglobin-based oxygen carriers can sustain oxygen carriage in acute anaemia, expand circulating volume and improve rheology to ischaemic tissues. When blood is limited, not available or not an option, the risks associated with these carriers must be weighed against the risk of death.

Supplies of blood are insufficient to meet the predicted future requirements of an ageing population because the elderly use a disproportionate amount of blood and blood products (Vamvakas, 1996). The donor pool is not growing sufficiently to meet demands, and new reasons to exclude donors continue to expand (National Blood Data Resource Center, 2005), resulting in a likely 4 million annual packed red blood cell (PRBC) unit shortfall in the USA by 2030 (Vamvakas, 1996).

These circumstances create an opportunity for an alternative oxygen carrier to PRBCs. Such alternatives would be useful in austere field and disaster response situations such as combat casualty, pre-hospital care of haemorrhaging patients, in field and disaster response locations remote from blood banks, and aboard ships and aircraft where acute blood loss requires immediate attention to prevent exsanguination (Patel et al, 2006). About one third of the US blood centres have 2 days or less supply of available blood (America's Blood Centers, 2008).

Medical reasons that blood is not an option include autoimmune haemolytic anaemia, antibody mismatch and other rare incompatibilities to available PRBC (Mullon et al, 2000; Mackenzie and Bucci, 2002). Surgical reasons for blood unavailability include inhospital acute blood loss when inadequate PRBC have been cross matched because of the recency of patient hospital admission or the suddenness and extent of bleeding because of surgical complications and limitation of in-hospital blood supplies (Levien et al, 2008). Ethical reasons for avoiding blood include patient refusal – the Jehovah's Witness population, estimated to number over 6 million worldwide, will not accept blood or blood products because of their religious beliefs (Hospital Liaison Committee, 2000). Reducing the use of PRBC to allow the predicted shortfall to be met, despite increased demand for blood, can be achieved by use of restrictive transfusion decision making (Hébert et al, 1999), coagulant technologies to avoid blood loss (Shander and Goodnough, 2006), blood salvage, acute normovolaemic techniques, so-called 'bloodless surgery' (Carless et al, 2006) and alternative oxygen carriers. Alternative oxygen carriers are one way of avoiding the most frequent 2–3-unit PRBC transfusion. If only one quarter of the current 250 000 US trauma patients had alternative oxygen carriers instead of PRBC, this would save 100 000 PRBC units each year and reduce allogeneic blood exposure by 20–25% (Mackenzie and Bucci, 2002).

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What alternative oxygen carriers are available?

There are three types of alternative oxygen carriers: haemoglobin-based oxygen carriers (HBOCs), haemoglobin solution encapsulated in microspheres, and perfluorocarbons. No encapsulated haemoglobin solutions or perfluorocarbon-based oxygen carriers are undergoing human trials or have commercial support in the USA or Europe (Winslow, 2003). Three HBOCs have completed or are undergoing phase 3 clinical trials: Hemopure (HBOC-201) (Biopure Corporation, Boston, MA), PolyHeme (Poly SFH–P) (Northfield Laboratories Inc, Evanston, IL), and Hemospan (maleimide polyethylene glycol haemoglobin, MP4) (Sangart Inc, San Diego, CA) (Center for Biologics Evaluation and Research, 2008). These three HBOCs differ in physical and physiological properties and source of haemoglobin (*Table 1*).

Hemopure (HBOC-201)

HBOC-201 has undergone a phase 3 trial in 688 elective orthopaedic surgery patients to determine whether HBOC-201 has efficacy and safety in avoidance of PRBC transfusion. Blood transfusion was avoided in 96.3% for 24 hours (337 of 350 patients randomized to HBOC-201) (Jahr et al, 2008). Therefore HBOC-201 would be efficacious for use until PRBC becomes available in field conditions or remote areas. PRBC avoidance was 67% by 1 week (234 patients), making it useful for an extended period when blood is not available and when blood is not an option. Avoidance of blood was 59.7% at 6-week follow up after surgery in those patients randomized to HBOC-201. When up to 10 units of HBOC-201 (the maximum allowed under the protocol before cross over to PRBC) were administered there was the same 1% mortality and 0.14 serious adverse events per patient in those who received HBOC-201 and PRBC (Mann-Whitney = 0.519, confidence limit (CL) = 0.48–0.56). There was no overall mortality difference in the intent to treat analysis. When there was a greater need for haemoglobin to reverse

Table 1. Comparison of various HBOCs currently in clinical development with red blood cells

Feature	Hemopure (HBOC-201) (Biopure Corporation)	PolyHeme (Poly SFH–P) (Northfield Laboratories Inc)	Hemospan (MP4) (Sangart Inc)	Red blood cells
Haemoglobin source	Bovine	Human	Human	Human
Type of modification	Glutaraldehyde polymerization	Pyridoxylation and glutaraldehyde polymerization	Polyethylene glycol conjugation	Not applicable
Average molecular weight (kDa)	64–500	150	90	Not applicable
Haemoglobin level (g/dl)	13	10	4.2	13
Methaemoglobin (%)	< 5.0	< 8.0	< 0.5	
Volume (ml)	250	500	250 or 500	
Oxygen pressure at 50% oxygen saturation (mmHg)	40	26–32	5–6	26–27
Oncotic pressure (mmHg)	25	23	49	25
Viscosity (cP)	1.3	2.1	2.2–2.5	5–10
Half life	19 hours	24 hours	43–66 hours	31 days
Shelf life	≤ 3 years (at 2–30°C)	≤1.5 years (at 4°C) ≤ 6 weeks (at 21°C)	≤ 3 years (frozen)	42 days (at 4°C) < 6 hours (at 21°C)
Clinical development status as of beginning of 2008	Regulatory approval for general surgery (South Africa)	Phase III trial in haemorrhagic shock following trauma completed enrollment (US)	Phase III trial in hip arthroplasty ongoing (Europe)	Not
	Regulatory filing for orthopaedic surgery (US)		Phase II trial in prostatectomy ongoing (US)	
	Phase II trial in cardiopulmonary bypass and aortic aneurysm reconstruction completed		Phase II trials in orthopaedic surgery completed (Sweden)	
	Phase II trials in trauma and percutaneous coronary revascularization enrolling (US)			

From Mackenzie and Shander (2008)

anaemia and patients crossed over to PRBC there was a higher rate of serious adverse events (0.63/HBOC patient *vs* 0.47/PRBC patient, *P*<0.0002) in patients randomized to HBOC-201 (Mann-Whitney = 0.605, CL = 0.55–0.66). Factors contributing to this greater incidence of serious adverse events included age >80 years, volume overload and under-treatment. Mortality rate was 16.1% *vs* 3.9% (HBOC-201 *vs* PRBC) in patients >80 years, whereas it was identical at 3.9% in those <80 years receiving either HBOC or PRBC. Because of the 19-hour half life, re-dosing with HBOC is needed and was inadequate in this trial. Total haemoglobin levels remained below those used to make the transfusion decision for the majority of the 6-day protocol-allowed time interval for infusion of alternative oxygen carriers (*Figure 1*).

Polyheme (Poly-SFH-P)

Polyheme has less non-proprietary published information than other HBOCs. Data from the Northfield web site (www.northfieldlabs.com/index.html), and a recent Food and Drug Administration (FDA) workshop (Gould, 2008) on safety of HBOCs, provide information on the phase 3 clinical trial recently completed in 719 trauma patients. These patients were treated at the site of injury and in one of 32 US level one trauma centres in 18 states. The control population received saline and PRBC (in the trauma centres), the treatment group received Polyheme, starting in the field and continuing for 12 hours in the trauma centres, up to a total of 6 units before cross-over to PRBC. The end point was survival at 30 days. Factors considered were age, gender, injury severity score, mechanism of injury (penetrating *vs* blunt), Glasgow Coma Scale score, systolic blood pressure and volume of crystalloid administered at point of randomization in the field.

This difficult to conduct study had 70 protocol violations (20%) in the Polyheme group and 56 (15%) in the control group, leaving only 586 patients eligible for data analysis of whom 279 received Polyheme. The study evaluated the potential to provide a benefit when blood transfusion was indicated but not available. The a priori outcome measures for efficacy and safety were a dual superiority and non-inferiority test of 30-day mortality in the modified intent to treat population. A difference between treatment and control of < zero and for non-inferiority an upper CL <7% were evidence of treatment benefit. Preliminary results indicate that in the primary modified intent to treat population (who received some Polyheme) the upper limit of CL was 7.3% and therefore exceeded 7%. However, in those patients who did not violate the protocol the upper CL was 5.8%, suggesting

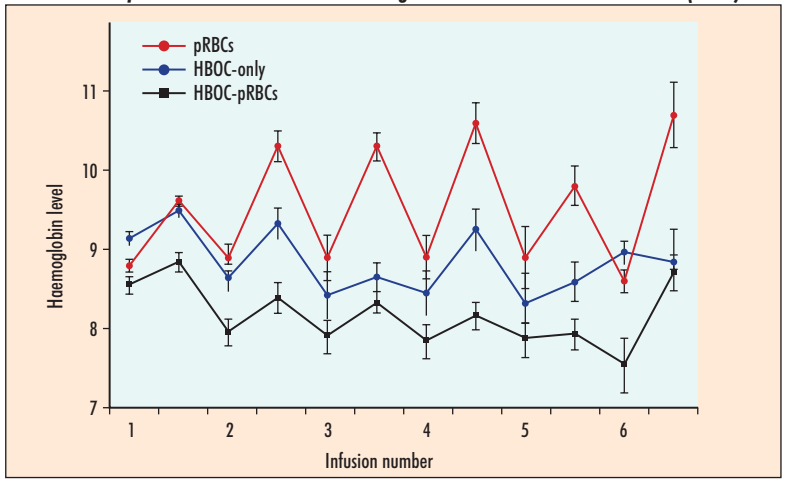
benefit. Peer reviewed publication will enable additional analyses and information to be obtained about this study. A study design limitation was that the control group got PRBC (more haemoglobin) and many of the treatment group patient data were confounded by receiving both HBOC and PRBC. It would have been a 'cleaner' analysis if Polyheme administration had stopped on trauma centre admission and Polyheme data were compared to pre-hospital crystalloid infusion.

Hemospan (MP4)

The third HBOC, Hemospan, is undergoing phase 3 trials in Europe (Keipert, 2008). It has major differences in physical and physiological properties from the other two HBOCs (*Table 1*) in molecular size, oxygen affinity, haemoglobin concentration, oncocity and prolonged circulatory retention time (half life) of 43–66 hours.

The theoretical benefits of the design of this HBOC include the prospect that the large molecule decreases diffusion, provides more viscosity and possibly less endothelial extravasation. The haemoglobin partial pressure of 5 mmHg when 50% saturated (P50) limits oxygen release in larger vessels and decreases oxidation, while the low haemoglobin concentration (4.3 g/dl) reduces toxicity and production cost. Increased oncocity makes Hemospan a good plasma expander. The manufacturer and investigators of this product believe that low P50 eliminates the vasoactivity caused by nitric oxide binding, thus functional capillary density is preserved in anaemia and shock allowing facilitated plasma oxygen diffusion in the capillaries of ischaemic tissues (Keipert, 2008).

Figure 1. The pre- and post-total haemoglobin values for packed red blood cell (pRBC) patients – the haemoglobin-based oxygen carrier 201 (HBOC-201) data are divided into patients that received HBOC-201 only and HBOC-201 plus subsequent pRBCs during the treatment period. The 12 data points represent preinfusion and postinfusion measurements for each of six infusions. The top horizontal dashed red line represents the median clinically significantly low haemoglobin concentration. The bottom horizontal dashed green line represents the average (n = 648) initial transfusion trigger. Data are presented from measurements of total haemoglobin obtained <30 minutes before or after clinical trial material infusions. The symbols are connected to facilitate comparison of the groups, however, the number of patients decreases with increasing infusion number. From Jahr et al (2008).



Hemospan completed a multi-site phase 2 clinical trial in Sweden of 59 patients undergoing major orthopaedic surgery, finding that Hemospan significantly (P=0.0003) reversed hypotension after spinal anaesthesia for primary hip arthroplasty (Olofsson et al, 2006). Phase 3 trials involve 36 European centres and will recruit 830 patients, 370 of whom will be in a protocol to prevent hypotension by administration of Hemospan at the time of spinal anaesthesia and a second unit administered when blood pressure starts to fall. A second protocol will recruit 460 patients to treat hypotension once it occurs after spinal anaesthesia. Enrollment is due to be complete shortly. It remains to be seen why and whether Hemospan is any more efficacious or safe, than the much less expensive crystalloid or colloid infusion, as the current standard of care with adrenergic drugs, for spinal anaesthetic hypotension.

Correction of acute anaemia with HBOCs

Anaemia increases complications and adverse events, especially in the elderly (Culleton et al, 2006). The haemoglobin concentration of HBOC-201 is 13 g/dl (similar to whole blood), whereas the haemoglobin concentration of PRBCs is about 2–2.5 times greater. PRBC are retained in the circulation for several weeks, making decrease in total haemoglobin as a result of metabolism unlikely compared to HBOC-201. Volume overload was historically a problem with whole blood transfusion and this appears to have re-surfaced as an issue with volume-expanding haemoglobin solutions (Greenburg, 2008).

HBOCs should be administered for anaemic patients in a dose sufficient to replace haemoglobin losses from bleeding (e.g. surgical, trauma, obstetric) and medical causes of anaemia (e.g. haemolysis, malaria) and the loss because of their half life. High oncotic pressure and low haemoglobin concentration limits the use of Hemospan to treat in people with anaemia (Keipert, 2008). Administration of HBOC-201 or Polyheme should begin if blood is not available, and the patient is continuing to lose blood, when haemoglobin falls below 8 g/dl (or higher if the patient has known myocardial disease or electrocardiographic evidence of ischaemia). Major therapeutic targets should be to optimize circulating volume (neither hypovolaemia nor overload), maintain total haemoglobin (total Hb = red cell Hb + plasma Hb from HBOC) in excess of 6 g/dl, by HBOC infusion at a rate consistent with the required increase in total haemoglobin, and to account for loss resulting from the half life.

What is the future for HBOCs?

The future use of HBOCs is in doubt because of a meta-analysis published in *JAMA* suggesting that HBOCs increase mortality and occurrence of myocardial infarction (Natanson et al, 2008). The methodology, conclusions and content of this meta-analysis and the accompanying editorial (Fergusson and McIntyre, 2008) have been criticized in six letters to the editor published in September 2008. These did not support the meta-analysis conclusions that

HBOCs (in general) increased the risk of death (164 *vs* 123 in controls) and myocardial infarction (59 *vs* 16 in controls), because of serious concerns about the methodology, errors and data included in the meta-analysis (Keipert et al, 2008; Levien et al, 2008; Lewis and Frost, 2008; Sarani and Gracias, 2008; Sauaia et al, 2008; Shander et al, 2008).

This meta-analysis included 13 prospective randomized clinical trials plus two proprietary reports (Polyheme) and one FDA review (HBOC-201) giving 16 prospective randomized clinical trials and 3711 patients considered, involving five different HBOCs. Two of the HBOCs included in the meta-analysis have ceased development and have no commercial support (HemAssist, Baxter Healthcare, Deerfield Il, USA, discontinued in 1998; Hemolink, Hemosol Inc., Mississauga, Ontario, Canada, discontinued in 2004). The HemAssist prospective randomized clinical trial in trauma patients, conducted more than 10 years ago, had a 40% mortality (Sloan et al, 1999), and when these data are removed from the meta-analysis there is no mortality difference. So the basis for statistical support for increased mortality is the result of one study of a product whose development has been discontinued.

The 16 prospective randomized clinical trials had varying methodologies, performed on heterogenous patients in different settings with various controls. There was no homogeneity among the trials pooled together for the meta-analysis. Individual trials should be weighted for correct pooling of data for a meta-analysis. This was ignored for the 22 separate HBOC-201 trials performed with different comparators (PRBC, crystalloid, whole blood and plasma) – these were erroneously considered as one large trial. Some of the so-called non-blood controls actually received blood and vice versa. High mortality (trauma Polyheme prospective randomized clinical trial) and low mortality (elective surgery) trials were pooled, making meta-analysis invalid. The meta-analysis also mixed the results of trials of HBOCs using as little as 50 ml with trials that used up to 10 units. If, as the *JAMA* article postulates, these HBOCs are more or less similar, then there was no consideration of a possible dose–response relationship. This illustrates the problem with the meta-analysis as the controls were different, the HBOC doses were different, the comparators included PRBC, whole blood colloids and crystalloids and the situations in which HBOCs were used were different.

Myocardial infarction data were obtained from both small and large studies, some with 2:1 randomizations. Pooling these results omitted the weighting necessary to include them in the meta-analysis. Many of the cardiac ischaemia events like myocardial infarction seen with HBOCs were related to under-treatment of anaemia in comparison to the comparator PRBC (Jahr et al, 2008). The accompanying editorial suggested that there was a place for HBOCs 'where blood was not accessible or available', but called for a moratorium on further clinical use of HBOCs (Fergusson and McIntyre, 2008). In South Africa, HBOC-201 has been approved for clinical use since 2001, because there are inadequate blood supplies. Data on the first 750 units administered to 336 patients, showing no deaths or myocardial infarctions directly related to HBOC-201 administration, were not included in the meta-analysis (Levien et al, 2008). Similarly, Hemospan data showing no deaths or myocardial infarctions in two prospective randomized clinical trials were not included, and errors were identified in the *JAMA* data used in the denominators of the Hemospan study (Keipert et al, 2008).

HBOCs are life saving in heavily bleeding patients when no blood is available or when death is imminent as a result of exceedingly low haemoglobin. The risks potentially associated with HBOCs must be balanced against potential benefits for specific conditions and settings. When blood is not available in emergencies or is not an option, the risks of HBOCs, whatever they are, must be weighed against the risk of death. An indiscriminate call for a moratorium on further trials is inappropriate when compassionate use programmes have shown that HBOCs are life saving (Mullon et al, 2000).

Advantages and disadvantages compared to blood

The advantages of HBOC over blood are the shelf life of 1–3 years that minimizes the cost and resources needed for re-stocking, compared to 6 weeks shelf life with blood. HBOCs require no cross match, some can be stored unrefrigerated at room temperature (2–30°C), whereas blood requires type and sometimes very lengthy cross-match and requires refrigerated storage.

HBOC could be used in remote locations, disasters, and for patients refusing, or unable for medical reasons to accept blood. Acellular HBOC solutions have improved rheology and facilitated cellular oxygen diffusion compared to blood. Both blood and HBOC can replete oxygen carriage and circulating volume deficits. HBOCs like erythropoietin can act as haemotinics and stimulate erythropoeisis. HBOCs can support anaemic patients after emergency surgeries or in intensive care when blood loss is large and unexpected so that blood is not readily available or in sufficient quantity. Blood transfusion has been associated with increased multi-organ failure (Marik and Corwin, 2008) and blood older than 2 weeks with an increased occurrence of respiratory, renal failure, septicaemia and death (Koch et al, 2008).

The disadvantages of HBOC over blood are that their half life means they have to be repeatedly dosed to maintain oxygen-carrying efficacy. Elevation in blood pressure can occur on and up to several hours after administration of some HBOCs. The vasoreactive mechanism includes binding of nitric oxide. However, the assumption in the *JAMA* meta-analysis that HBOCs act as a class because of nitric oxide binding has not been proven. Hemospan has less vasoreactivity than other HBOCs. Blood does not elevate blood pressure other than by circulating volume expansion. Other known side effects of HBOCs include scleral and skin discolouration, which are the result of metabolism of HBOC and are often mistakenly interpreted as jaundice. Various gastrointestinal effects occur, per-

haps as a result of nitric oxide binding on smooth muscle. Increases in liver enzymes and lipase result from interference with protein clearance in the reticulo-endothelial system (Jahr et al, 2008). Laboratory tests dependant on colourimetric analyses show interferences. Plasma haemoglobin minimizes the optical beam transmission of oximeters, similar to that of fingernail polish, and affects pulse oximetry and mixed venous oximetry read-outs. True haemoglobin oxygen saturation levels are obtained by co-oximetry. HBOC infusion may result in methaemoglobinaemia, especially when red cell haemoglobin is low (<5 g/dl) because methaemoglobinaemia reductase levels are reduced proportional to haematocrit. Methaemoglobinaemia elevation above 10% is managed with daily vitamin C or methylene blue 1 mg/kg by intravenous infusion.

Conclusions

HBOCs have side effects including vasoreactivity that may cause myocardial infarction, stroke and death in some patients. When blood is not available for acute anaemia, or is not an option for medical, technical or ethical reasons, HBOC's side effects are less hazardous than those of not giving blood. The ubiquitous 3-unit PRBC transfusion can be replaced by HBOC, with adequate haemoglobin concentration, in otherwise healthy young haemorrhaging patients. HBOCs sustain oxygen carriage and avoid transfusion errors until PRBCs are available, providing a potential solution to the predicted shortfall in PRBCs. **BJHM**

Table 1 *is reproduced by kind permission of OpenJournals Publishing and* Figure 1 *is reproduced by kind permission of Lippincott Williams & Wilkins. Conflict of interest: Dr CF Mackenzie has received funding from Biopure Corporation, manufacturers of Hemopure, both as a site principal investigator in the HEM–0115 trial and as a consultant.*

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KEY POINTS

- Whether to use haemoglobin-based oxygen carriers or not is determined by risk tolerance.
- When blood is indicated but not available or in sufficient quantity in emergencies or when blood is not an option, the risks of haemoglobin-based oxygen carriers, whatever they are, must be considered against the risk of complications of acute anaemia, including death.
- In remote locations, disasters, combat field locations and to meet the need for future predicted blood shortages haemoglobin-based oxygen carriers are beneficial because they need no cross-match, and have a 1–3-year shelf life, some without refrigeration.
- Haemoglobin-based oxygen carriers with adequate haemoglobin levels can safely replace the ubiquitous 2–3 unit packed red blood cell transfusion.
- Some haemoglobin-based oxygen carriers facilitate oxygen diffusion in ischaemic tissues without vasoreactivity and have prolonged sustained volume repletion.

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