

Fluids as oxygen carriers and the potential role in trauma resuscitation

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Patients with major trauma present a challenge, often using large quantities of banked blood both at the time of injury and during their hospital stay. Blood transfusion is not without risk and is associated with high costs; it is immunosuppressive, rendering patients more susceptible to infection. In the western world, banked blood is fully screened and relatively safe; the same is not true in parts of the developing world, where high rates of HIV carriage make blood transfusion a risky undertaking. Additionally, blood transfusion as a vector for transmission of illnesses such as prion disease is a distinct possibility, for both the developed and developing world alike. The introduction of artificial blood substitutes would ameliorate some risk and also remove the cost of extensive blood testing. For trauma outside hospital, blood substitutes could compete directly with fluid resuscitation as donated blood is not usually available. Patients with prolonged transport times would appear to be the most obvious beneficiaries and volume expansion, along with improvement in oxygen-carrying capacity would be the ultimate goal. All clinicians confronted with the need for transfusion of homologous blood would welcome the development of a safe and reliable alternative to red blood cells in order to ensure oxygen transport to the tissues. However, even though research on red cell substitutes started more than 100 years ago, even now none of the heavily investigated compounds based on haemoglobin or perfluorocarbons has been released in Europe or the USA for routine clinical use.

Key words: red cell substitutes; oxygen carriage; perfluorocarbons. haemoglobin-based oxygen carriers (HBOCs)

Introduction

The indication for transfusion of red cells in significant trauma is severe haemorrhage. The aim of red cell transfusion is to improve the oxygen supply to the tissues by raising the oxygen content of the blood, according to the equations shown in Table 1.

The dissolved oxygen contributes little under normal circumstances but can be elevated significantly when breathing under pressure (hyperbaric conditions).

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The effect of hyperbaric oxygen (HBO) in improving tissue oxygenation has been explored widely and is used therapeutically in some circumstances. The equations in Table 1, in relation to dissolved oxygen hold true at one standard atmosphere (1ATA: 1 ATA = 760 mmHg = 1.013 bar) or sea level pressure. HBO therapy involves the use of oxygen under pressure greater than that found on the surface of the earth at sea level. Only a limited amount of oxygen is dissolved in blood at normal atmospheric pressure but under hyperbaric conditions it is possible to dissolve sufficient oxygen to meet the usual requirements of the body. In such cases oxyhaemoglobin will pass from the arterial to the venous side unaltered because the oxygen dissolved in solution will be more readily utilised than that bound to haemoglobin (Hb) (Shirley and Ross, 2001a¹). At sea level the plasma oxygen concentration is 3 mL/L.

Table 1 Calculation of oxygen delivery (Adapted from Jones, 1995)

Oxygen delivery = cardiac output × arterial oxygen content

Arterial oxygen content = (Hb concentration × % saturation × 1.34) + dissolved oxygen

Oxygen delivery (DO₂) is represented by the following equation: $DO_2 = [(Hb \times 1.34) \times (1.00)] + [(PaO_2 \times 0.0031)]$

where, Hb = haemoglobin (gm/dL), 1.34 = oxygen carrying capacity of each gram of Hb, 1.00 = 100% Saturation of arterial blood, PaO₂ = arterial pressure of oxygen in mmHg, 0.0031 = the Bunsen solubility coefficient of dissolved oxygen per mmHg.

Example: So for a patient with Hb of 15 g/dL, saturated at 100% and PaO₂ of 100 mmHg

$DO_2 = [(15 \times 1.34) \times (1.00)] + [(100 \times 0.0031)] = 20.1 + 0.31 = 23.2 \text{ mL O}_2 \text{ 100 mL}$.

(Therefore, there is 0.31 mL of dissolved oxygen per 100 mL, ~3 mL/L).

Tissues at rest require about 60 mL of oxygen per litre of blood flow (assuming normal perfusion) to maintain normal cellular metabolism, although requirements vary between tissues. At a pressure of 3 atm (304 KPa) dissolved oxygen approaches 60 mL/L of plasma, which is almost sufficient to supply the resting total oxygen requirement of many tissues without a contribution from oxygen bound to Hb. Oxygen at 300 KPa increases oxygen tension in arterial blood to nearly 270 KPa and in tissue to about 53 KPa. This improves the cellular oxygen supply by raising the tissue–cellular diffusion gradient. This has advantages in situations such as carbon monoxide poisoning or in severe anaemia where difficult cross-matching or religious belief prevents blood transfusion (Leach *et al.*, 1998). The method of HBO administration in large fixed pressure chambers with all the attendant risks, make it an impractical solution for improving oxygen delivery in trauma patients with severe haemorrhage (Shirley and Ross, 2001b²). Currently available resuscitation fluids are effective in restoring blood pressure by volume expansion but are clearly not a substitute for oxygen-carrying blood. Conventional reasoning indicates that this limitation relates to oxygen-carrying capacity reaching the threshold where it can no longer satisfy the metabolic demand of the body. At this point blood transfusion is essential to sustain life. Some experimental, systemic and microvascular findings obtained using polyethylene glycol conjugated albumin solutions in extreme haemodilution and shock resuscitation in animal models have suggested successful resuscitation from levels of haemorrhage previously considered fatal without the use of blood but as yet this has translated into clinical practice (Tsai *et al.*, 2008).

The transfusion of blood (or plasma or red cells) involves many hazards some of which may be reduced or avoided but cannot ever be completely eliminated.

Transfused blood can require about 24 h to reach full oxygen transport capacity due to 2,3-diphosphoglycerate (2,3-DPG) depletion. Potential shortages and perceived issues with the safety of blood have strengthened interest in the search for alternatives to blood. Over two million units of red cells were transfused in the UK in 2006. There were approximately 400 incidents related to errors with transfusion (an increase on 2005) and four transfusion-related deaths (SHOT Report, 2006). Although the blood supply in the UK is very safe, this is not the case everywhere; blood transfusion is the second largest source of new HIV infections in Nigeria. In certain regions of South Africa as much as 40% of the population has HIV/AIDS, and thorough testing is not financially feasible. A disease-free source of blood substitutes would clearly be beneficial in these regions (Table 2).

The development of oxygen-carrying fluids

There have been two broad approaches to constructing a fluid capable of transporting and delivering oxygen in the human circulation:

- Perfluorocarbons (PFCs)

PFCs are synthetic fluorinated hydrocarbons (similar to teflons) that increase dissolved oxygen in the fluid phase of the blood without binding the oxygen molecule and hence cause a linear relationship between the amount of O₂ dissolved and PO₂ in contrast to the native Hb (which has a sigmoid shaped O₂ dissociation curve). PFCs are biologically and chemically inert and possess high gas dissolving properties; they are not miscible with water and have to be emulsified prior to use.

Table 2 The desirable properties of a red cell substitute (Adapted from O'Connor, 2004)

(1)	Non-antigenic
(2)	Inert
(3)	Stable, with ability to maintain haemodynamic stability and arterial pH
(4)	No renal toxicity
(5)	Oxygen-carrying capacity comparable to that of Hb/whole blood
(6)	Oxygen-binding curve comparable to that of Hb
(7)	Sigmoid curve is desirable for unloading
(8)	Devoid of side effects
(9)	Favourable or no effect on blood viscosity
(10)	Long shelf-life, no preparation before use
(11)	Long duration of effect (weeks to months)
(12)	Inexpensive (but economically viable to manufacture)
(13)	Storage at room temperature
(14)	Rapid infusion possible
(15)	Large permissible dosing
(16)	Avoids, rather than delays transfusion

- Haemoglobin-based oxygen carriers (HBOCs)

Haemoglobin-based oxygen carriers (HBOCs) are derived from humans, animals, or artificially via recombinant technology. However, pure Hb separated from red cells cannot be used since it causes renal toxicity. It can be treated to avoid this, but it still has undesirable oxygen transport characteristics. Various other steps are needed to form Hb into a useful and safe oxygen carrier. These may include cross-linking, polymerisation and encapsulation. These are needed because the red cell is not a simple container for Hb, but a complex entity with many biomolecular features.

Perfluorocarbons

PFCs are chains of 8–10 hydrocarbon molecules where the hydrogen has been replaced by fluorine. They have a short half-life of 12–18 h but have delayed clearance preventing multiple doses in a short space of time. On-loading and off-loading of oxygen with PFCs is not possible at narrow physiological ranges of PO_2 like Hb but only at very high FiO_2 . PFCs facilitate enhanced micro-circulatory O_2 delivery by virtue of their small molecular size and non-binding to O_2 . With sophisticated technology, it is possible to generate a stable perfluorocarbon emulsion with

exceptionally small particles (median diameter $<0.2\ \mu\text{m}$) PFCs are excreted unchanged by the lungs after passing through the reticuloendothelial system (RE) which at times can cause RE cell blockade. PFCs have to be administered as lipid emulsions as they are immiscible in water. Pure PFCs at 760 mmHg dissolve 40 mL of O_2 per 100 mL of PFC, approximately twice that of arterial blood. The first approved PFC was Fluosol-DA-20, manufactured by Green Cross of Japan. It was approved by the Food and Drug Administration (FDA) in the US 1989. Because of limited success, complexity of use and side effects, it was withdrawn in 1994.

Oxygent (Alliance Corp. San Diego) a stable 60% (wt/vol.) emulsion of perfluorooctyl bromide (perflubron) has been developed using egg yolk phospholipid as the sole emulsifying agent. Under normal arterial and venous oxygen tensions, Oxygent can unload as much as 1.3 mL oxygen per 100 mL; however, the oxygen delivery capacity of Oxygent is still $<30\%$ of normal blood (5 mL O_2 /100 mL blood at 15 g Hb/dL) and may still require oxygen-enriched air breathing to ensure adequate oxygen delivery. Unlike Fluosol-DA, Oxygent has a longer intravascular half-life ($t_{1/2} = 9\ \text{h}$ at 4 kg/kg in rats) and is excreted from the body in about 4 days (compared to months for some components of Fluosol-DA). The longer circulation time and reduced total body clearance time of Oxygent are considered more desirable for most clinical uses with an average particle size of 0.16–0.18 μm diameter. That this blood substitute is completely man-made gives it certain distinct advantages over blood substitutes which rely on modified Hb, such as unlimited manufacturing capability, the ability to be heat-sterilised and the PFCs' efficient oxygen delivery. Oxygent is compatible with all blood types and has a shelf-life of approximately 2 years. It is indicated for the of oxygen delivery inpatients at risk of tissue oxygen deficit due to surgical blood loss, transient anaemia or ischaemia (Spahn, 1999). Baxter has the licence to market Oxygent in the EU but there is no information concerning possible launch dates or trials in the UK. Oxygent has been evaluated in 20 clinical studies involving 1500 subjects. A phase III study of Oxygent in patients undergoing various general surgical procedures found a significant reduction in the need for blood donation (Spahn *et al.*, 2002). Enrolment in a Phase III

trial in cardiac surgery was suspended following an unexpected difference in the number of stroke incidents between the control and treated groups.

Some reports suggest that Perftoran, a 10% perfluorocarbon emulsion, was approved by the Russian Ministry of Health for clinical use in 1996. Seemingly, Perftoran had been tested in over 2000 patients by the year 2000 for various clinical conditions including severe anaemia, haemorrhagic shock, cerebral ischemia and cardiac surgery. It is claimed that the use of Perftoran reduced ischaemic/hypoxic damage, improved haemodynamics and decreased oedema. According to the company website this compound has been used on Russian troops, suffering injury in Chechnya, as well as recently (2006) in patients undergoing cardiac surgery. Many details including exact dosage and nature of any adverse effects are not readily available (Kim and Greenberg, 2004).

Interesting work has been done by Kacmarek *et al.* (2006) with a modified PFC for use in partial liquid ventilation for patients with adult respiratory distress syndrome (ARDS), as an oxygen carriage medium. When compared to conventional ventilation, the use of PFCs in either high or low doses conferred no outcome benefit and its use is not recommended in this group of patients.

Haemoglobin-based oxygen carriers

HBOCs have been studied since 1934, when purified bovine Hb was administered to study animals and 1949, when purified human Hb was infused into anaemic patients. Early approaches to the development of oxygen carriage involved the use of stroma-free Hb solutions. These solutions did not require blood typing or cross matching and could be stored for long periods. It was found that removal of Hb from red cells leads to the loss of 2,3-DPG and diminished oxygen-delivery characteristics. Furthermore, Hb tetramers tend to dissociate into dimers, which have a short intravascular persistence (due to rapid renal excretion) and a nephrotoxic action secondary to precipitation in the proximal tubule (Baron, 1999). Development of Hb-based blood substitute was pursued vigorously by the military as a means to have an oxygen-carrying plasma expander available for battlefield use in the US (Table 3).

Table 3 Methods of preparation of HBOCs (Jahr *et al.*, 2007)

(1)	From natural stroma-free Hb (human, bovine)
(2)	Hb cross-linking: [alpha]-[alpha] and [beta]-[beta]
(3)	Binding to macromolecules cross-linked with diaspirin or raffinose
(4)	Polymerisation with glutaraldehyde
(5)	Encapsulation
(6)	Microencapsulated Hb
(7)	Hb lipid vesicles
(8)	Biodegradable polymer Hb nanocapsules
(9)	Genetic engineering
(10)	Natural or modified Hb
(11)	Bacteria
(12)	Yeasts

To date two cross-linked Hb products have been subject to phase III clinical trials.

- (i) Diaspirin cross-linked Hb (DCL Hb; HemAssist, Baxter Healthcare, Deerfield, IL) is a human tetrameric Hb product that uses a diaspirin bridge to cross link the alpha subunits. An in-hospital trial of diaspirin cross-linked Hb failed to demonstrate a survival benefit after injury and instead documented increased mortality (Sloan *et al.*, 1999) This was a first generation product that was affected adversely by a high percentage of tetrameric Hb (>30%). An European trial using the same product in the pre-hospital setting, comparing resuscitation with saline or DCLHb, did not identify increased mortality in patients treated with DCLHb (Kerner *et al.*, 2003). A multi-centre study evaluating the use of DCLHb in cardiac patients demonstrated that infusion of up to 750 mL of DCLHb allowed 19% of post-operative cardiac surgery patients who otherwise would have received allogeneic blood to avoid exposure to red cell transfusions. However, given the eventual transfusion of most DCLHb recipients, the higher incidence of adverse events in the DCLHb group, and the solution's relatively short plasma persistence (half-life <24 h), the routine use of DCLHb for transfusion avoidance in this population was not supported (Lamy *et al.*, 2000).

Further work targeted reducing tetramerism and a great deal of work on DCLHb was initially performed by the US Army. They contracted Baxter

to produce DCLHb (HemAssist) on a large scale. However, due to significant adverse effects associated with it, the US Army discontinued further development of this compound. The results of the clinical trials showing high mortality and adverse incident rates lead to Baxter terminating further development and testing.

- (ii) Hb raffimer (Hemolink; Hemosol Inc, Ontario, Canada), is a poly Hb product for use in conjunction with intra operative autologous donation which was tested with initially encouraging results. However, trials were stopped in 2003, due adverse side effect profiles and production of Hemolink has been terminated (Greenberg and Kim, 2004).

Three polymerised Hbs have also been developed and tested in Phase III trials:

- (i) Polystroma-free Hb (Poly SFH-P; PolyHeme Northfield Laboratories, Evanston, IL) is a glutaraldehyde cross-linked, pyridoxylated human poly Hb, in which all tetrameric Hb is removed to decrease side effects. The therapeutic benefit of PolyHeme has been compared directly with that of allogeneic red blood cells in the treatment of acute blood loss. Patients given PolyHeme were able to maintain total Hb concentration and also reduced the amount of donated blood the patient needed by nearly half. A total of 171 patients received rapid infusion of 1–2 units ($n=45$), 3–4 units ($n=45$), 5–9 units ($n=47$) or 10–20 units ($n=34$) of PolyHeme. Hb was adequately maintained (mean, 6.8 ± 1.2 g/dL) because of plasma Hb added by PolyHeme. The 30-day mortality was 25.0% (10/40 patients) compared with 64.5% (20/31 patients) in historical control patients at these Hb levels. This was a non-randomised study but seemed to indicate that PolyHeme increases survival at life-threatening Hb levels by maintaining total Hb in the absence of red cell transfusion (Gould *et al.*, 2002)

The first U.S. Phase III trial of Hb-based oxygen-carrying resuscitative fluid in which treatment began in the pre-hospital setting has been completed. Thirty-two level one trauma centres throughout the USA participated in the trial (total 720 patients). Treatment began at the scene of injury, continued in

the ambulance during transport, and for up to 12 h post-injury or six units in the hospital. The trial is designed to evaluate PolyHeme against standard fluid resuscitation, followed by blood transfusion in-hospital when required. The primary endpoint is survival at 30 days. Published results are awaited but the fact that the trial reached completion is an encouraging sign.

- (ii) Hb-based oxygen carrier 201 (HBOC 201; Hemopure, Biopure Corporation, Cambridge, MA, USA) is a glutaraldehyde cross-linked bovine poly Hb that has the advantages of a readily available source, a naturally high P50, a long shelf-life and limited side effects. It also uses glutaraldehyde for cross-linking of Hb. The side effects profile does not appear to be limiting and clinical testing is ongoing. It uses the naturally low affinity of bovine Hb for oxygen as a rationale for its use; thereby aiding off-loading. Biopure Corporation have completed a worldwide phase III clinical trial in human patients on Hemopure in elective surgery. In April 2001, Hemopure was approved for use in South Africa for acute anaemia in surgery patients, and it is the first HBOC approved for human use. Concern over administration of large quantities of bovine proteins to humans which may stimulate antibody production and fears over transmission of bovine spongiform encephalopathy (BSE) have been counteracted by using a dedicated herd of cattle, free of this disease and heat treatment of Hb to ensure sterility (Kotur, 2004).
- (iii) MalPEG (Hemospan, Sangart Inc., San Diego, CA): Successful Phase II trials with this maleimide-treated polyethylene glycol-modified haemoglobin (PEG-Hb) compound have been completed in elective orthopaedic patients undergoing major surgery (Olofsson *et al.*, 2006). A multi-centre randomised, double-blind controlled Phase III study of the efficacy and safety of Hemospan compared with colloid (Voluven) for prevention of perioperative hypotension in patients undergoing primary hip arthroplasty with spinal anaesthesia is now recruiting patients. Hemospan has properties of reduced extravasation from the circulation, thereby avoiding the hypertensive side effects of some HBOCs.

Hemospan also has a higher oxygen affinity than blood and the most other HBOCs (Ferguson *et al.*, 2008). An examination of the basis for the vasopressor effects observed with the earlier Hb-based oxygen carriers led to the insight that several properties of Hb solutions, such as their diffusion coefficient for oxygen, viscosity and colloid oncotic pressure, are important determinants of efficacy (Winslow, 2003). With this in mind Hemospan seems to have been developed to combine oxygen carriage with enhanced plasma expansion. It is surprising therefore that a trial involving resuscitation in the emergency situation has not been designed.

Potential benefits of red cell substitutes in clinical practice

Blood substitutes have been considered for several reasons; including the disease transmission risks in the developing world discussed above. In addition, there is currently no practical way to test for prion-transmitted diseases in donated blood. Demand for blood continues to rise, in the US, climbing by between 6 and 8% as an aging population requires more surgical interventions. Artificial oxygen carriers are universally compatible with all blood types. There is no need for typing or cross-matching, preventing the risk of ABO incompatibility associated with human error which still occurs despite checking of packed red cells in 1:34 000 units (Tappenden, 2007). In battlefield scenarios, it is often impossible to administer rapid blood transfusions and military medical care would benefit from a safe, easy way to manage blood supply, reducing the need for the use of emergency donor panels from amongst the colleagues of injured troops. In the civilian pre-hospital environment, where transfer times may be prolonged, an easily accessible alternative to blood would have a place. Transfused blood is currently more cost effective, but there are reasons to believe this may change. For example the cost of blood substitutes may fall as manufacturing becomes refined. Blood substitutes can be stored for much longer than transfused blood, and can be kept at room temperature. Most Hb-based oxygen

Table 4 Perceived benefits of red cell substitutes in clinical practice

(1)	Avoids the issues of cross-matching blood
(2)	Avoid the issues of infection transmission
(3)	Overcomes logistics of refrigeration and storage
(4)	Avoidance of immune suppression
(5)	Exploit physical properties of substitutes
(6)	Reduced costs per unit transfused
(7)	Potential clinical applications
(i)	Resuscitation (field, emergency department, military)
(ii)	Haemodilution for elective surgery
(iii)	Ischaemic heart disease and angioplasty
(iv)	Cardioplegia for donated organ preservation
(v)	Anaemias (e.g. sickle cell)
(vi)	Red blood cell incompatibility
(vii)	Acute lung injury

carriers in trials today carry a shelf-life of between 1 and 3 years, compared to 42 days for donated blood, which needs to be kept refrigerated. Blood substitutes allow for immediate full capacity oxygen transport; transfused blood can require about 24 h to reach full oxygen transport capacity due to 2,3-DPG depletion. The extended life of current artificial Hb carriers allows for stock-piling for emergencies, trauma, disasters and warfare. Data from Israel suggests that the in planning for terrorist attacks, an adequate blood supply should be 1.3 red cell units and 1.0 component per patient if all casualties are included or 6.7 red cell units and 4.5 components per severe or moderately injured patient. Their experience illustrates the advantages of a comprehensive programme for managing blood operations in emergency situations. A coordinated national programme is in place ensure instant access to defined blood bank inventories that facilitate sufficient supply in times of surge during major incidents and reduce wastage (Shinara *et al.*, 2006). Clearly red cell substitutes would partly reduce the pressure put on such systems (Table 4).

Disadvantages of HBOCs

The HBOCs have a high oxygen-carrying capacity, significant oncotic effect and a long shelf-life. However, they are also rapidly cleared (plasma half-life within 24 h) and may induce systemic and pulmonary hypertension through vasoactivity.

Three possible mechanisms proposed for the hypertension are:

- Hb is a scavenger of nitric oxide thereby increasing the systemic vascular resistance
- Stimulation of endothelin release causing rise in vascular tone
- Direct stimulation of alpha adrenergic receptors by Hb

Some of these effects have been observed in animal trials using HBOC-301 (a veterinary version of HBOC-201) in resuscitation from traumatic brain injury with severe haemorrhage compared with Ringers lactate solution. The use of HBOC-301 was superior to the standard care group; eliminating the need for red cell transfusion later and permitting earlier weaning from ventilation (King *et al.*, 2005).

It is not obvious that an optimal plasma expander has already been devised; and blood is probably not optimal in this context. Moreover, the design of a blood substitute that has the properties of native blood in the circulation may be unattainable, particularly because it is not possible to reproduce the biomechanical characteristics of blood (Intaglietta, 2008). The pro-inflammatory effects of artificial blood substitutes are an area of concern, although some of the early work with PolyHeme and levels of Interleukins (IL-6 and IL-8), seemed to suggest a possible attenuation of this response in early trauma resuscitation (Moore *et al.*, 2005).

Use of HBOCs in trauma

The most compelling indication for HBOCs is the scenario in which stored red cells are unavailable. This potential benefit for military use has largely driven the development of HBOCs, but there are also a number of key applications in civilian trauma care. Most obvious is the role in pre-hospital care, particularly for extended transport times. However, there are also remote hospitals in which large quantities of stored blood are not available (or stocks are rapidly depleted when multiple casualties are encountered). There have been well-designed animal models that strongly suggest pre-hospital low-volume resuscitation with HBOCs can save lives. Despite the appeal, the scientific design and

ethical conduct of clinical trials to establish efficacy of HBOCs when red cells are unavailable remain a challenge. Northfield Laboratories came under scrutiny in the USA, for their PolyHeme Phase III trial they conducted in 32 level one trauma centres across the country. The controversy arose from the fact that the participants in this study were incapable of giving their consent due to the nature of their injuries. Even though the consent process was sanctioned ethically by the FDA as necessary emergency research, patients' rights groups have challenged the conduct of the study (Moore *et al.*, 2005). Hemopure is about to be tested in a Phase II study will be a single-centre, randomised, single-blind, parallel-group, standard therapy-controlled, variable dose study of HBOC 201 administered to trauma subjects with bleeding or potential for bleeding who require standard fluid therapy for treatment of hypoperfusion. The type and incidence of adverse events and serious adverse events attributed to the study drug will be analysed. The extension of the use of Hemopure in a trauma population in South Africa would be of clear benefit, where up to 60% of male trauma victims are HIV positive and blood donation is not widely taken up (Goosen *et al.*, 2003).

Future directions

Hb-vesicles (HbV) are artificial oxygen carriers that encapsulate concentrated Hb solution within a thin lipid membrane producing a liposomal suspension. Animal tests have clarified the efficacy of HbV as an alternative to red cell transfusion. The results of ongoing HbV research have indicated further development of HbV is viable, with the expectation of its eventual use in humans (Sakai *et al.*, 2008).

Stem cells as a means of producing an alternate source of blood have also been studied. Large-scale *in vitro* production of mature human blood cells using haematopoietic stem cells has been reported by Giarratana *et al.* (2005) and may represent the first significant steps in this direction. Blood cells produced in culture possessed the same Hb content and morphology as native red cells. The red cells produced had a near-normal lifespan, when compared to native red cells; an important characteristic

that current Hb-based blood substitutes do not possess. The major obstacle with this method of producing red blood cells is cost. At the moment, the complex three step method of producing the cells would make a unit of these red blood cells prohibitively expensive. However, the study is the first of its kind to demonstrate the possibility of producing red blood cells which closely resemble native red blood cells on a large scale.

Recently, Baxter has developed a second generation HBOC using recombinant technology. This new compound, rHb2.0, has a haem pocket that has been re-engineered to reduce affinity for nitric oxide (to decrease vasopressor effects), while preserving the favourable O₂ kinetics. The actions of this novel substance in conditions designed to mimic severe perioperative blood loss in pigs, have been studied. The results show that rHb2.0 is a safe and effective resuscitation solution in these experimental conditions that simulate haemodilution and severe peri-operative blood loss. The data were submitted to the FDA in the USA in preparation for a human clinical trial (Proctor, 2003; Malhotra *et al.*, 2003).

Summary

The most promising products in this field at present appear to be the Hb-based oxygen carriers of PolyHeme, Hemopure and Hemospan. The relative safety and efficacy of PolyHeme and Hemopure has been demonstrated by clinical trials, and Hemopure has been licensed for commercial use in South Africa. At present, there are no randomised controlled trials comparing both products directly. Furthermore, there is no evidence on long-term follow-up after transfusion of either, effects of long-term or repeated use. Currently the Hemopure dose is limited by the manufacturer to seven units and coupled with its short half-life, making its application for abolishing allogenic red cell transfusion entirely limited but potentially important as an off-the-shelf, ready to use universally compatible, life saving product when blood is not available. The outcome of the large PolyHeme pre-hospital trauma trial and the planned hemopure trial will hopefully lead to the development of a clinically, more widely available artificial oxygen carrier (Tappenden, 2007). The results of the first Hemospan Phase III trial seem some way off at present.

Oxygen-carrying blood substitutes, even if widely available would not eliminate the use of human blood, which performs various functions besides oxygen transport. (platelets, clotting factors and white cell function) Alternatives to red blood cell transfusions (blood substitutes) are designed to overcome known limitations. New dangers of blood transfusions are also being described, including inflammatory agents contained in stored blood that may prime leukocytes and cause adverse immunomodulatory effects (Winslow, 2002; Goodnough and Shander, 2003). In various situations, particularly early trauma resuscitation, there would be potential advantages over human blood, which are worth pursuing as the technology develops and improves.

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