Blood substitutes
Haemoglobin therapeutics in clinical practice

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Early approaches to the development of oxygen carriers involved the use of stroma-free hemoglobin solutions. These solutions did not require blood typing or crossmatching and could be stored for long periods. In addition, a variety of methods have been developed in chemically modifying and stabilizing the hemoglobin molecule. Several hemoglobin therapeutics are now in clinical trials as temporary alternatives to blood or as therapeutic agents for ischemia. The various hemoglobin products under development are derived from three principal sources: human, bovine and genetically engineered hemoglobin. Diaspirin cross-linked hemoglobin (DCLHb), administered at doses ranging from approximately 20–1000 ml, has been investigated in a number of clinical trials in patients undergoing orthopedic, abdominal aortic repair, major abdominal surgery, cardiac surgery and in critically ill patients with septic shock. In several studies, DCLHb was effective in avoiding the transfusion. However, Baxter Healthcare Corporation (Chicago, Illinois, USA) stopped the development of DCLHb after two unsuccessful trials in trauma patients. Bovine polymerized hemoglobin has also been extensively studied. Several phase II and phase III trials have been performed with this product in hemorrhagic surgery, cardiac surgery and vascular surgery, but data have not yet been published. Hemoglobin therapeutics could provide an important new option as an alternative to blood transfusion. Furthermore, they may be able to provide an immediate on-site replacement for traumatic blood loss, prevent global ischemia and organ failure, treat focal ischemia, and provide effective hemodynamic support for septic shock-induced hypotension.

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Introduction

A number of oxygen-carrying alternatives to blood have been in clinical evaluation since Amberson [1] administered haemoglobin–saline solutions in clinical trials. Fourteen patients received an injection of a haemoglobin–saline solution. Of these, five patients with secondary anaemia due to haemorrhage or infection received multiple injections, and three of these patients showed clinical improvement, consisting of increased haemoglobin and haematocrit values and reticulocytosis. The use of these early haemoglobin solutions had a number of drawbacks, however, including signs of renal impairment, resulting from short intravascular persistence and impurities of the haemoglobin introduced during preparation. The first generation of haemoglobin therapeutics has addressed the infectious disease risk, storage, stability and typing issues. These solutions have the potential to augment the oxygen-carrying capacity of patients' red blood cells temporarily and help avoid allogenic transfusion.

Sources of haemoglobin

Early approaches to the development of oxygen carriers involved the use of stroma-free haemoglobin solutions [2]. These solutions did not require blood typing or crossmatching and could be stored for long periods. It was discovered, however, that removal of haemoglobin from red cells leads to the loss of 2,3-diphosphoglycerate and diminished oxygen-delivery characteristics. Furthermore, haemoglobin tetramers tend to dissociate into dimers, which have a short intravascular persistence (due to rapid renal excretion) and a nephrotoxic action secondary to dimer precipitation in the proximal tubule [3]. Therefore, in recent years, a variety of methods have been developed to prevent these problems by chemically modifying and stabilizing the haemoglobin molecule. Several haemoglobin therapeutics are now in clinical trials as temporary alternatives to blood or as therapeutic agents for ischaemia. The various haemoglobin products under development are derived from three principal sources: human, bovine and genetically engineered haemoglobin.

The rationale for using human haemoglobin is related to the reduced risk of causing allergic or other immune reactions in the recipient. The viral risks associated with blood transfusions, already reduced by extensive donation screening tests, are greatly minimized during the haemoglobin therapeutic manufacturing process by rigorous viral-inactivation procedures. Some chemically stabilized

DCLHb = diaspirin cross-linked haemoglobin; HBOC = haemoglobin-based oxygen carrier.

haemoglobin solutions, such as diaspirin cross-linked haemoglobin (DCLHb or HemAssist™, Baxter Healthcare Corporation, Chicago, Illinois, USA) are subjected to ultrafiltration and heat treatment during the manufacturing process to inactivate viruses [4]. Other human haemoglobin-based products are currently being developed, including pyridoxylated and glutaraldehyde polymerised haemoglobin (PolyHeme™, Northfield Laboratories Inc, Evanston, Illinois, USA), o-raffinose cross-linked and polymerised haemoglobin (HemoLink™, Hemosol Inc, Toronto, Canada) and pyridoxylate haemoglobin polyoxyethylene (Apex Corporation, Plainfield, Illinois, USA).

Bovine haemoglobin is readily available and, potentially, has a lower cost as a raw material than human haemoglobin [5,6]. Like human haemoglobin, however, bovine haemoglobin requires modification to be clinically effective. Furthermore, infectious agents present in animal blood, such as the bovine spongiform encephalopathy prion, are not as well characterized and therefore are more difficult to detect than those in human blood. Animal-sourced products must also be subjected to rigorous purification procedures, because contamination with animal-derived proteins may cause allergic reactions in humans. Bovine haemoglobin is usually derived from protected herds in countries without bovine spongiform encephalopathy incidence, however. In addition, the specific purification techniques used may be able to eliminate the protein responsible for bovine spongiform encephalopathy transmission, even though the nature of the protein is unknown. A bovine haemoglobin product was recently approved for veterinary use in canines (Oxyglobin™, Biopure Corp, Cambridge, Massachusetts, USA). A second bovine haemoglobin, glutaraldehyde polymerized bovine haemoglobin (Hemopure™, Biopure Corp), is in a phase III human clinical trial in Europe. This product was also tested in patients in sickle cell crisis without producing side effects [7]. A third bovine-derived product, polyethylene-glycol conjugated bovine haemoglobin (Enzon Inc, Piscataway, New Jersey, USA) is also under investigation.

Recombinant technology is also being investigated to engineer oxygen-carrying solutions. These techniques allow flexibility in product design, enabling haemoglobin molecules to be modified to address specific clinical needs. In addition, many of the potential side effects of bovine-sourced products, or indeed the sourcing complications associated with human red blood cells, may be avoided by using recombinant technology. Purification requirements for recombinant products are quite high, but the source material is theoretically unlimited. The only recombinant haemoglobin subjected to clinical trials is obtained by genetic engineering from *Escherichia coli* and is a modified human haemoglobin tetramer cross-linked with a glycine bridge between the α-subunits (Optro™, Somatogen Inc, Boulder, Colorado, USA) [8]. Pilot studies of tolerance were carried out on 24 volunteers [9]. No renal, hepatic, or pulmonary toxicity was noted. There was no renal excretion, and no significant variation in either systolic or diastolic arterial blood pressure. Fever, higher than 38°C, occurred 3–8 h after infusion in 12 individuals, with headache, myalgia and chills. No further episodes of fever were noted after improving the purification process. Further clinical development of this product has been stopped, however, pending new generations of recombinant haemoglobin solutions. The possibility of obtaining haemoglobin from transgenic tobacco plants was recently reported [10].

Cross-linked haemoglobins

The most extensively studied of the modified haemoglobins, is diaspirin cross-linked haemoglobin (DCLHb) or HemAssist™ (Baxter Corp.). DCLHb is produced using 3,5-dibromosalicylate to form a four-carbon fumarate bridge between the two α-subunits of the haemoglobin molecule. The stabilization of DCLHb during manufacture not only permits heat treatment, but also induces the haemoglobin to assume a low-affinity conformation similar to that in the human red blood cell in the presence of 2,3-diphosphoglycerate. Consequently, DCLHb has similar oxygen-carrying properties to those of fresh blood. However, DCLHb has a slightly lower affinity for oxygen than fresh blood (right shifted oxygen dissociation curve), which allows it to release oxygen more readily to tissues compared with red blood cells.

DCLHb has been extensively studied in preclinical studies with excellent results [11–15]. In animal models of haemorrhagic shock, DCLHb was more effective than infusion of large volumes of lactated Ringer's solution in restoring arterial blood pressure and tissue oxygenation [16]. In preclinical studies, the safety profile was also excellent without undesirable effects such as antigenicity, complement or white blood cell activation or renal toxicity. An early and sustained increase in mean arterial pressure associated with a decreased heart rate was frequently reported [11,17–19]. This pressure increase is dose dependent and is easily controlled using antihypertensive agents. Three mechanisms may contribute to the vasopressive effect of DCLHb: nitric oxide scavenging [20], stimulation of endothelin release [20], and sensitization of α-adrenergic receptors [21].

DCLHb, administered at doses ranging from approximately 20 to 1000 ml, has been investigated in a number of clinical trials in patients undergoing orthopaedic surgery, abdominal aortic repair and major abdominal surgery [22], and in critically ill patients with septic shock [23]. In these completed studies, involving more than 700 patients, safety assessments indicated that DCLHb was well tolerated. In a small study [22], DCLHb (750 ml) effectively eliminated the need for any transfusions of

blood for a period of 7 days in one-third (four out of 12) of patients undergoing elective hip arthroplasty with high blood loss. A larger trial in post bypass cardiac surgery patients requiring transfusion was undertaken to determine the efficacy of DCLHb in reducing or preventing the postoperative use of blood transfusions [24]. In that multicenter study, 209 patients were enrolled when it was determined that they required transfusion after bypass. The patients randomly received up to three doses (total 750 ml) of DCLHb or up to three units of packed red blood cells within 24 h after removal from bypass. DCLHb was effective in avoiding the transfusion of packed red blood cells in 59% of recipients on the day of surgery, when all patients in the control group received allogeneic blood. On the day after surgery, 39% of patients receiving DCLHb had still avoided receiving blood. At 7 days after surgery, 19% of the DCLHb-treated patients had completely avoided a transfusion of packed red blood cells. The most common adverse reactions were transient and nonserious, and consisted of hypertension, yellowing of the skin (reported as jaundice), haemoglobinuria and hyperamylasaernia. There were no clinical sequelae from these events. Two nonreported multicenter studies have been conducted in trauma patients. These trials have been stopped because of excessive mortality in the treated group. Baxter subsequently stopped the development of DCLHb.

Bovine haemoglobin

Bovine polymerized haemoglobin has also been extensively studied. Standl *et al* [25] compared the effects of stored red cells, freshly donated blood and ultrapurified polymerized bovine haemoglobin [haemoglobin-based oxygen carrier (HBOC)] on haemodynamic variables, oxygen-transport capacity and muscular tissue oxygenation after acute and almost complete isovolaemic haemodilution in a canine model. The results showed a higher oxygenation potential of HBOC than with autologous stored red cells because of more pronounced oxygen extraction. Horn *et al* [26] investigated the effect of HBOC on skeletal muscle tissue oxygenation in comparison with hetastarch during nearly complete arterial stenosis. After isovolaemic haemodilution with Ringer's lactate solution to a haematocrit of 25%, a 95% artificial stenosis of the popliteal artery was established. In both groups oxygen delivery and oxygen consumption of the muscle decreased in parallel to the decreasing blood flow during arterial stenosis. In contrast, oxygen extraction ratio increased after infusion of HBOC and was higher after the second application when compared with hetastarch-treated animals. During stenosis tissue oxygenation was decreased and remained low after administration of hetastarch, but increased to nearly baseline values after HBOC treatment, suggesting that increased oxygen extraction in the HBOC group is associated with improved skeletal muscle tissue oxygenation during severe arterial stenosis.

In a whole-animal sheep model, Vlahakes *et al* [5] showed in awake sheep that extreme haemodilution to a haematocrit of $2.4 \pm 0.5\%$ was only tolerated when a polymerized bovine haemoglobin solution was used, but not in animals treated with hydroxyethyl starch devoid of oxygen-carrying capacity. All of the animals that survived acute haemodilution also survived the following 25 days, without evidence of renal or hepatic dysfunction. Similar results were achieved recently when the blood of anesthetized dogs was haemodiluted to a haematocrit of $2.0 \pm 1.8\%$ using a polymerized bovine haemoglobin solution [27]. Even at this extremely low haematocrit, the dogs were haemodynamically stable, there was no evidence of lactic acidosis and there were no histologic signs of ultrastructural destruction in liver and kidney.

Several phase II and III trials have been performed with this product in haemorrhagic surgery, cardiac surgery and vascular surgery, but data have not yet been published.

Future developments

The properties of haemoglobin therapeutics include effective transport and delivery of oxygen, much like red cells. Unlike the red cell, however, the haemoglobin molecule in solution appears to have positive effects on the microcirculation of key tissues and organs, with easier penetration into capillaries because of low molecular size and low solution viscosity. These properties and the vasopressor effects suggest a number of potential therapeutic applications.

Considering the emergency use setting, haemoglobin therapeutics may allow emergency department personnel to avoid time-consuming and expensive cross-matching. Haemoglobin therapeutics may allow more effective on-scene or emergency department resuscitation of trauma patients and reduced risk of organ failure secondary to the hypoxic insult.

Furthermore, haemoglobin therapeutics can be formulated to exert a colloid osmotic pressure which, combined with the vasopressor activity, allows a rise in blood pressure without any detrimental effects on regional perfusion. As a result, haemoglobin therapeutics may be capable of treating the hypotension, while maintaining regional perfusion, in patients with septic shock.

Other potential indications for haemoglobin therapeutics include stroke and myocardial infarction, perfusion of tumours to enhance cancer therapy, and sickle cell anaemia.

Haemoglobin therapeutics under investigation are relatively short-lived compared with transfused red cells, placing potential limits on their future applications. Beyond these approaches, a range of other solutions that may have longer intravascular persistence and could, therefore, be closer to the concept of blood substitutes are being

developed. Such initiatives include liposome-encapsulated haemoglobin, synthetic heme-embedded lipid microspheres, and other oxygen-carrying artificial red cells.

Conclusion

Preclinical studies have shown haemoglobin therapeutics to be as effective as blood and more effective than standard colloid or crystalloid solutions for resuscitation from haemorrhagic and septic shock. In the early phases of clinical trials, these solutions have been well tolerated, with only minor side effects. Haemoglobin therapeutics could provide an important new option for the treatment and prevention of ischaemia resulting from hypoperfusion. Furthermore, they may be able to provide an immediate on-site replacement for traumatic blood loss, prevent global ischaemia and organ failure, treat focal ischaemia, and provide effective haemodynamic support for septic shock-induced hypotension.

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