Synthetic blood products: science fiction or coming to an ICU near you?

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Despite 128 years of intense research, the development of an oxygen-carrying plasma expander remains a Holy Grail for clinical practice, academia and the pharmaceutical industry. Almost concurrently with the development of blood transfusion as a therapy, there was research to find an alternative that avoids the adverse effects of transfusion.[1] Allogeneic blood transfusion carries many risks, including infectious disease transmission, transfusion reactions, delayed wound healing, transfusion-related acute lung injury, immunomodulation, and the potential risk of neoplasia recurrence.[2,3] The logistic challenges inherent in blood cross-typing and lack of portability limit the use of allogeneic transfusion in trauma and in more remote situations. Furthermore, blood supply is limited, with increasing shortages, and the issue of safety of "old" stored blood-bank blood compared with freshly donated blood-bank blood may also be significant.

In 1949, Amberson et al[4] were the first to report the infusion of cell-free haemoglobin in humans. They infused small volumes of haemoglobin solutions, and reported a pressor response, and "resuscitated" a woman with severe postpartum haemorrhagic shock; however, she later died with acute renal failure. The authors noted that haemoglobin solutions appeared to cause hypertension and bradycardia, and were not yet ready for clinical use. This early realisation that haemoglobin solutions are not only red blood cell substitutes, but also have many other properties, including haemodynamic effects related to their oncotic and nitric oxide scavenging properties, has seen the slow evolution of progressive generations of red blood cell substitutes. Many different solutions have been developed and tested to varying degrees in animals and humans.

Characteristics of the ideal red blood cell substitute are shown in Table 1. Current research in blood substitutes centres on perfluorocarbon emulsions, which increase the amount of non-haemoglobin-bound oxygen. Other studies have utilised various modifications of haemoglobin to reduce toxic effects and to replicate the oxygen transport properties of native red blood cells.

Table 1. The ideal red blood cell substitute

The ideal red blood cell substitute has the following characteristics:

- Immediate availability
- Effective oxygen-carrying capacity
- Effective volume expansion
- No infection risk
- No immunological risk
- A long intracirculation half-life
- Minimal or no side effects
- Non-toxic
- No special requirements for storage
- A long shelf-life
- Universal compatibility, eliminating the need for cross-matching
- Acceptability to all religious persuasions
- Cost effectiveness
- Readily available supply

Perfluorochemicals

The perfluorocarbon emulsions were the first of the blood substitutes to undergo clinical trials. Perfluorochemical (PFC) liquids are hydrocarbon molecules of eight to 10 carbon atoms in length, where the hydrogen atoms have been replaced by fluorine atoms. These liquids have several unique properties: they are immiscible with water, have a density about twice that of water, are chemically inert, and have nearly 20 times the solubility for gases of water. Because of their chemical inertness and high solubility for oxygen, emulsions of PFCs in normal saline have been studied over the past 30 years as oxygen-carrying colloids that may temporarily supplement oxygen transport in lieu of erythrocytes.

As PFCs carry oxygen by direct solubility, their contribution to oxygen content, like that of plasma, is directly proportional to the arterial oxygen tension (Pa_{O_2}); they require a high Pa_{O_2} (> 300 mmHg) to be effective. Another limitation of these emulsions is that they are removed rapidly from the vascular space by the reticuloendothelial system, with a half-life in the range of 12–18 hours.[5] They are inexpensive and easily manufactured, but because of their oxygen-loading characteristics, patients who receive them must inspire high concentrations of supplemental oxygen. Unfortunately, perfluorocarbons also need to be refrigerated during storage, which, combined with their relatively short half-life in the circulation, makes them more suitable for use in hospitals than in off-site emergency resuscitation.[6]

Fluosol-DA (manufactured by the Green Cross Corporation, Osaka, Japan) was tested in patients with acute haemorrhage who refused blood for religious reasons.[7] Its

performance was disappointing in that setting. However, it was eventually licensed for use in percutaneous transluminal coronary angioplasty for perfusion of the arterial bed distal to the balloon.[8] Adverse effects associated with complement activation and cytokine release have been controlled with the use of new formulations containing smaller emulsion particles. However, an influenza-like syndrome, attributed to macrophage-mediated phagocytosis of the emulsion, and an unusual sequestration of about 20% of circulating platelets are commonly observed with PFCs.

The lessons learned with Fluosol-DA resulted in the development of two new perfluorocarbons: Oxygent (Alliance Pharmaceutical Corporation, San Diego, Calif, USA)[9] and Oxyfluor (HemaGen/PFC, Waltham, Mass, USA),[10] which have a higher capacity for respiratory gases and do not require special preparation before use — as necessary for Fluosol-DA. At present, only Oxygent is still in clinical use, primarily as an adjunct to acute normovolaemic haemodilution. The rationale for its use in this setting is that the replacement of whole blood withdrawn before surgery with an oxygen carrier would permit more extreme haemodilution and increase the efficacy of this technique for minimising allogeneic blood transfusion.[11]

Haemoglobin-based oxygen carriers

The idea of using purified haemoglobin as a possible universal substitute for red blood cells has been around for nearly a century, based on haemoglobin's unique oxygen-binding property and the perceived lack of antigenicity. Each normal adult haemoglobin molecule has a tetrameric structure, comprising two alfa and two beta polypeptide chains. Each unit is linked by an iron ion (Fe^{2+}) to a haem molecule, where the oxygen is fixed. The haemoglobin used to generate haemoglobin-based oxygen-carrying solutions comes from three potential sources, each offering advantages and disadvantages: human blood, bovine red cells and recombinant haemoglobin solutions.

Outdated stocks of donated human blood can be used to manufacture haemoglobin solutions, but, ironically, the limited supply of human blood could limit large-scale production. Bovine red cells are readily available and have the advantage of possessing a lower affinity for oxygen than human haemoglobin (bovine haemoglobin is devoid of 2,3-diphosphoglycerate, and therefore its P_{50} remains around 30 mmHg, both within and outside the red blood cell[12]). However, concerns remain about the antigenicity of bovine haemoglobin and transmission of infections, such as bovine spongiform encephalitis.

Recombinant haemoglobin solutions have been produced and have the advantage of oxygen-binding and dissociation characteristics similar to those of human blood. Specific functional and physical properties can be introduced into these molecules, modifying their stability, oxygen affinity and half-life. The risk of disease transmission is virtually eliminated, although the risk of contamination during the manufacturing process exists. These solutions also may be acceptable to Jehovah's Witness patients, who refuse blood for religious reasons. However, the technology involved is expensive, and the biggest problem with these solutions is large-scale, cost-effective production.

Limitations of stroma-free haemoglobin

Although haemoglobin removed from the protective environment of the red blood cell membrane (stroma-free haemoglobin) continues to carry oxygen, it encounters two significant problems. When it is removed from the red blood cell, low concentrations of 2,3-diphosphoglycerate cause the oxyhaemoglobin dissociation curve to shift left. The haemoglobin oxygen affinity then becomes too high for effective oxygen release and tissue oxygenation. The P_{50} of human blood is around 27 mmHg, while the P_{50} of a pure haemoglobin solution may be as low as 10 mmHg.

The second problem with stroma-free haemoglobin is the rapid dissociation of the tetramers into their component alfa and beta dimers, which are rapidly filtered by the kidney. Renal filtration may then result in a short intravascular half-life and the potential for severe nephrotoxicity.[12]

A further problem encountered in the development of haemoglobin-based oxygen carriers has been vasoconstriction. Many early reports of haemoglobin infusion were characterised by severe hypertension, bradycardia and abdominal pain.[1,2,4] Current theory on vasoactive properties suggests that a decrease in nitric oxide concentration, caused by its interaction with haemoglobin, is responsible. Alternative theories claim too much oxygen is delivered directly to the subendothelium, resulting in an autoregulatory vasoconstrictor reflex. Yet another theory argues that oxidation of soluble haemoglobin could result in haem loss, free radical formation, loss of reactive iron, and oxidation of lipids. This cascade of events could then culminate in endothelial stress causing vasoconstriction.[13–15]

Modified stroma-free haemoglobin

Solutions of unmodified stroma-free haemoglobins are thus clinically unsuitable. When stroma-free haemoglobin is extracted from human or bovine red blood cells, it must be chemically stabilised to be therapeutically useful.[13] Various strategies have been used to effect this stabilisation. Intramolecular modification to cross-link the two alfa (α–α) or two beta (β–β) subunits stabilises the association of the two α–β dimers. This cross-linking not only prevents tetramer dissociation, but also reduces the affinity of haemoglobin for oxygen. 2-Nor-2-formylpyridoxal 5′-phos-

phate (NFPLP) has been successful in this regard. Polymerisation of haemoglobin via intermolecular cross-linking with dialdehydes, such as glutaraldehyde and glycoaldehyde, effectively increases the size of molecules through the formation of haemoglobin oligomers. This increase in size prevents the rapid excretion of the molecule, prolonging the haemoglobin intravascular half-life. Conjugation is the covalent binding of haemoglobin to a biocompatible polymer, such as a polysaccharide. Multiple polyethylene glycol chains have been added to the haemoglobin protein to increase the molecule's size. This also increases plasma half-life and reduces nephrotoxicity by minimising renal excretion. Finally, encapsulation of haemoglobin within lipid vesicles, using a solution of phospholipids, aims to re-create the natural properties of erythrocytes in the absence of blood group antigens. Manipulation of membrane properties may also render the encapsulated haemoglobin more functional.[1,16]

Trials of haemoglobin-based oxygen carriers

Two haemoglobin-based oxygen carriers are currently undergoing, or have completed, US Food and Drug Administration Phase II trials: Hemopure (HBOC-201, haemoglobin glutamer-250 (bovine); Biopure Corporation, Cambridge, Mass, USA) and PolyHeme (polymerised pyridoxylated haemoglobin; Northfield Laboratories, Evanston, Ill, USA). In April 2001, Hemopure was approved for use in South Africa for acute anaemia in surgery patients, and indeed it is the first haemoglobin-based oxygen carrier approved for human use.[13]

Enthusiasm needs to be tempered by the knowledge that several similar products have been withdrawn from further production following adverse study results. In 2004, a Phase IIb trial of Hemolink (Hemosol Ltd, Mississauga, ON, Canada) in cardiac surgery was suspended because of an increased incidence of adverse cardiac events in Hemolink-treated patients compared with the control group.[17,18] In 1998, Baxter Healthcare halted further development of diasprin cross-linked haemoglobin (HemAssist) after trials in patients with stroke and trauma demonstrated a significant increase in mortality in the treatment groups.[19]

Further trials of haemoglobin-based oxygen carriers are currently underway. Patients with trauma are being enrolled in a Hemopure trial in South Africa,[20] and there are additional Hemopure studies involving coronary artery bypass graft surgery patients in the United Kingdom, Greece and South Africa.[21] Hemospan (Sangart Inc, San Diego, Calif, USA) is being tested in the setting of hypotension in hip arthroplasty patients, and results should soon be available.[22]

Natanson et al recently conducted a systematic review and meta-analysis to assess the safety of haemoglobin-based oxygen carriers in nearly 4000 patients between 1980 and 2008.[23] Based on their analysis of available data, the authors found a 30% increase in the risk of death and nearly a threefold increase in risk of myocardial infarction with the pooling of all haemoglobin-based oxygen carrier trials. These results suggest there is still substantial work to be done before a safe and effective haemoglobin-based oxygen carrier becomes universally available.

Conclusions

The search for the ideal oxygen-carrying blood substitute has been long and complex. To date, the perfect agent has not been formulated, but the journey has provided valuable insights into the obstacles and requirements for the successful development of such a carrier. Blood substitutes are scientific fact, but their arrival at an intensive care unit near you is still a little way off.

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