

THE RISKS OF BLOOD TRANSFUSIONS AND THE SHORTAGE OF SUPPLY LEADS TO THE QUEST FOR BLOOD SUBSTITUTES

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A number of factors have combined to drive the interest in developing blood substitutes. These include the time-dependent decrement in stored blood biochemistry, the general shortage of the blood supply, and public awareness of the risks associated with allogeneic transfusions.

Current literature on different blood substitutes was reviewed. The aim of this article is to help the reader understand the necessity of blood substitutes and to briefly

describe blood substitutes that are in clinical trials.

The need for oxygen-carrying blood substitutes is the driving force in multiple clinical trials. More research is needed to develop alternatives to allogeneic blood transfusion that are free of complications.

Key words: Blood substitutions, Hemolink, Oxygent, perfluorocarbon.

Approximately 12 million units of blood are transfused in the United States each year.¹ Ninety years ago, blood could be stored for only 6 days (Table 1). Today, a unit of blood can be stored for 42 days; around 2% of the units become outdated. Blood requires cross-matching to decrease the possibility of transfusion reactions, consuming time and money resources. The incidence of human immunodeficiency virus transmission by blood transfusions is around 1 in 1 million to 1 in 2.5 million units of blood, and the risk of hepatitis C is about 1 in 100,000 to 1 in 350,000 units.²

Among blood's many functions is the carriage and transport of oxygen. This is the basis of the development of oxygen therapeutics. This feature is one of the many important blood functions and is one of the hardest to develop synthetically.

A large effort went into the development of blood substitutes in World War II when the need for blood products exceeded the amount available.³ Episodic shortages in the blood supply and the multiple dan-

gers associated with transfusions have led to research directed toward developing blood substitutes. Lines of research include the development of perfluorocarbon (PFC) emulsions and different types of free hemoglobin solutions such as hemoglobin-based oxygen carriers (HBOCs). The ideal blood substitute is one that can be used as a safe alternative to blood, transports and releases oxygen readily, and can be used in emergency situations such as in hemorrhagic shock, severe anemia, or extreme normovolemic dilution (Table 2). Blood substitutes also would be suited to patients who refuse blood transfusions for religious or cultural reasons.⁴ Blood substitutes could be used to treat conditions in which we have deemed it unreasonable to use banked blood or for which there is no therapy. The purpose of this article is to introduce the reader to blood substitutes currently under consideration.

What are blood substitutes?

Blood has a variety of functions, including oxygen and carbon dioxide carriage, transfer of nutrients,

Table 1. Comparison between perfluorocarbon, hemoglobin-based oxygen carrier solutions (HBOCs), and packed blood cells

Attributes	Packed blood cells	HBOC products	Perfluorocarbon
Shelf life	6 wk	24-36 mo	2 y
Refrigerator	Yes	No	No
Blood type	Yes	No	No
Size, μm	7-8	< 0.007	0.2
Risks	Disease Compatibility	Increased blood pressure	Low platelet count

Table 2. Ideal blood substitute characteristics

- Low cost
- Stable at room temperature
- Has the same or better oxygen-carrying capacity as blood with good unloading and offloading characteristics
- No liver or kidney toxic effects
- Easy to use
- Nonantigenic
- Long shelf life
- Similar pH and viscosity to blood
- Does not cause vasodilatation or vasoconstriction (ie, no interaction with nitric oxide)
- Absence of metabolism and active metabolites
- Nonreactive with oxygen

coagulation, immune defense, hormone transport, homeostasis, and a sophisticated ability to rid the body of toxins and wastes. Blood substitutes are designed to replace blood components; one such product is stroma-free hemoglobin that is based on human or bovine hemoglobin. Another product is the synthetically produced PFC emulsion. Blood substitutes are intended primarily as oxygen transporters and volume expanders. Ideally, they should possess the following characteristics: (1) rapid uptake and delivery of oxygen, (2) long shelf life, (3) nonantigenic, (4) removal of carbon dioxide, (5) stable over wide range of temperatures, (6) minimal cost, and (7) nontoxic, with minimal side effects. The oxygen-carrying capacity of blood is the focus of research into blood substitutes because the loss of this function is most rapidly fatal.³

Blood substitute research also has been fueled by the progressive, time-dependent decrement in banked blood performance. For example, red blood cell membranes lose flexibility, leak potassium, and become prone to hemolysis. 2,3-Diphosphoglycerate, crucial to oxygen offloading, becomes undetectable at 42 days of storage (personal communication, B. Johnston, April 2003).

A number of blood substitutions are under evaluation and development. Research and clinical trials in alternatives to red blood cell transfusions are currently underway in many settings. The progress is slow, but many are optimistic that blood substitutes will soon be available for general clinical use.⁵ If a good blood substitute is developed, it will have potential benefits such as wide availability, long shelf life, and freedom from viral and bacterial contamination.²

Review of literature

• *PFC emulsions.* Fluosol-DA, 20%, was the first PFC emulsion used in clinical practice. The conditions of some Jehovah's Witnesses improved, while the conditions of others did not. Fluosol was approved by the US Food and Drug Administration for use in angioplasty, in which it improved outcomes and resulted in decreased myocardial infarctions. The PFC emulsions were introduced to the medical field in 1966, with the experiment using a mouse submerged in a liquid of oxygenated PFC emulsions and surviving.

The PFC molecule is based on a linear or cyclic carbon backbone that contains fluorine and other halogens with a molecular weight of 450 to 500 d. PFCs are purely synthetic compounds that are biologically and chemically inert and capable of dissolving large volumes of oxygen and carbon dioxide.⁶ PFC emulsions are approximately 0.2 μm in diameter and have the ability to enter the microcirculation. The extremely small size of PFC emulsions allows them to negotiate narrow conduits that otherwise would prevent red blood cell access to maintain capillary perfusion during vasoconstriction and thrombosis. The PFC emulsions represent a family of compounds directed at providing tissue oxygenation.⁶

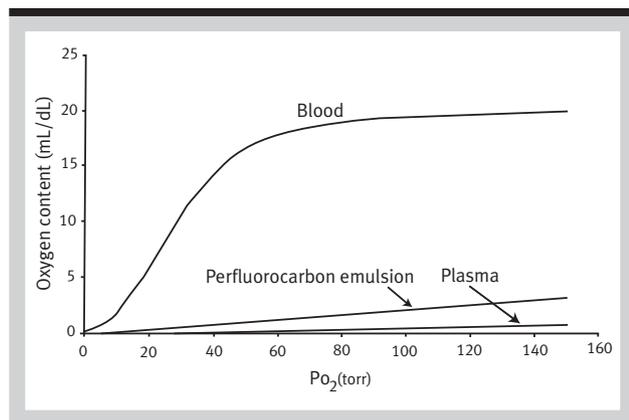
Carbon dioxide generated in the tissues diffuses freely into the venous plasma and is picked up by the PFC emulsions. The PFC emulsions readily unload carbon dioxide down a concentration gradient in the lungs for exhalation.⁶

The PFC emulsions have a linear PO_2 function compared with the sigmoid binding curve of hemoglobin. The amount of oxygen carried in PFC emulsions is in proportion with the PO_2 , that is, as the PO_2 increases, the amount of oxygen carried by PFC emulsions increases (Figure 1). This characteristic somewhat limits the use of PFC emulsions because positive-pressure ventilation to maintain high oxygen tension is needed. On the other hand, PFC emulsions can be extremely useful in cardiopulmonary bypass surgery, in which the ability to expose the PFC emulsion to 100% oxygen through the oxygenator is available to load the emulsion with large amounts of oxygen.⁷

The PFC emulsions have a half-life of 6 to 12 hours, making them useful as a bridge to blood transfusion; in addition, there is the potential for extending this half-life with newer PFC generations.² Pure PFC emulsions have been used in liquid ventilation (complete and partial liquid ventilation) to improve oxygenation during acute respiratory distress syndrome in premature newborns, demonstrating improved oxygenation in the first 4 days of treatment and improved lung compliance.⁶

Second-generation Fluosol did not have a much

Figure 1. Comparison between blood, perfluorocarbon emulsion, and plasma as a fraction of P_{O_2}



higher solubility for oxygen than blood plasma. Oxygen is transported by the PFC molecule without chemical bonding and is based exclusively on physical solubility, a characteristic that considerably facilitates the unloading of oxygen at the tissue level.

The physical characteristics of PFC emulsions (approximately 0.2 μm in diameter) permit only small intravenous doses at one time to avoid reticular endothelial system overloading.⁴ Flulike symptoms associated with the phagocytosis of the emulsion particles by the macromonocyte system have been seen in some recipients.⁷ These flulike symptoms generally

decrease within 24 hours. Newer formulations have been developed to reduce this side effect.⁸ The synthetic nature of PFC emulsions eliminates the possibility of infectious disease transmission. Table 3 gives a detailed list of advantages and disadvantages of PFC emulsions. In contrast with stroma-free hemoglobin solutions, PFC emulsion droplets do not have vasoconstrictor effects.

Oxygent is a second-generation PFC emulsion that is in clinical trials. It is an emulsion of perflubron (perfluorooctyl bromide) and is being used as a bridge to preserve oxygen transport until red blood cell transfusions can be given.² It can be stored at refrigerated temperature for up to 2 years.⁷ Another second-generation product is Oxyfluor, which has properties similar to those of Oxygent and is stable at room temperature for more than a year.

Sphan et al⁹ evaluated the use of Oxygent, a PFC product, in acute normovolemic hemodilution (ANH) in noncardiac surgery. The study was a single-blinded, randomized study performed in 34 centers across Europe from November 1998 to June 2000. The study demonstrated that ANH and treatment with PFC emulsions resulted in a decrease in the need for allogeneic transfusion. Patients were assigned randomly to 1 of 2 groups. Group 1 received PFC emulsion. Subsequently, this group underwent ANH to achieve a hemoglobin concentration of 8.0 ± 0.5 g/dL at a frac-

Table 3. Advantages and disadvantages of blood substitutes

	Advantages	Disadvantages
Perfluorocarbon emulsions	<ul style="list-style-type: none"> • Synthetic; excellent oxygen and carbon dioxide carriage eliminates the possibility of transfusion of human pathogens • Low cost • Prolonged shelf life • No vasoconstrictive effects • Small size 	<ul style="list-style-type: none"> • Flulike symptoms • The need for a high F_{iO_2} • Low oxygen-carrying capacity at physiological P_{O_2} • Rapid plasma clearance
Modified hemoglobin substitutes	<ul style="list-style-type: none"> • Increase the oxygen content of blood • Functions at physiologic P_{O_2} • Physiology of hemoglobin is well understood • Free of infection risk • No need for type and crossmatch for compatibility • Small size 	<ul style="list-style-type: none"> • Hypertension (ie, scavenging nitric oxide) • Renal toxic effects • Sepsis • Esophageal dysfunction • Abdominal discomfort • Cytotoxic effects

F_{iO_2} indicates fraction of inspired oxygen.

tion of inspired oxygen (FiO_2) of 1.0. At a hemoglobin concentration of 8.0 g/dL, the patients in this group received 1.8 g/kg of PFC emulsion. If the hemoglobin concentration dropped to 6.0 g/dL, another 0.9 g/kg of PFC emulsion was given. ANH blood was transfused if the hemoglobin concentration dropped to 5.5 g/dL. Group 2, the control group, received allogeneic erythrocytes from preoperative autologous donations or allogeneic erythrocytes if the hemoglobin concentration dropped to 8.0 ± 0.5 g/dL. The FiO_2 in this group was 0.4. The target for both groups was a hemoglobin concentration at the end of surgery of 8.5 g/dL.

The study revealed that at 24 hours, the number of patients in group 1 was higher than in group 2 with regard to avoiding all types of transfusions. On the day of discharge, there was no significant difference in the number of patients receiving blood and blood components in the 2 groups. However, the platelet count for patients in group 1 was significantly lower.

Group 1 had a significantly higher incidence of postoperative ileus than group 2. Mortality was 4% in group 1 and 2% in group 2, differences that did not reach statistical significance. The sepsis and infection rates were the same in both groups. A higher incidence of cardiovascular system adverse effects (ie, hypertension) was noted in group 1. The authors attributed this adverse effect to the mandatory retransfusion of all remaining ANH blood and to possible insufficient experience with the ANH process (autologous blood is harvested during surgery to be reinfused toward the end of the procedure) that some institutions may have experienced. Another contributing factor mentioned by the authors was the degree of hypovolemia experienced in group 1 vs group 2.

This study revealed the potential for PFC emulsions as an alternative to blood transfusion. Additional studies are underway that will help clarify and define the role of PFC emulsions as oxygen and carbon dioxide carriage alternatives to blood.⁹

• *Hemoglobin substitutes.* Hemoglobin substitutes are hemoglobin derivatives that are being researched as blood substitutes. These products do not have antigenic features and do not require compatibility testing. Stowel et al⁶ administered hemoglobin solutions to anemic patients, but the research was stopped owing to the chemical instability of the solutions. Subsequent work led to purification of hemoglobin substitutes directed at eliminating the toxic effects associated with stroma-free hemoglobin. The earlier hemoglobin substitute preparations had the disadvantage of having a high affinity for oxygen that impeded unloading of oxygen at the cellular level. A new approach is under development that uses chemically

modified cellular hemoglobin, with improved offloading capacity.¹⁰ The HBOC solutions are prepared from outdated human red blood cells and bovine red blood cells and differ markedly from PFC synthetic constitution. Because many problems are seen when free native hemoglobin substitutes are present in plasma, various forms and techniques are being used to modify hemoglobin substitutes.

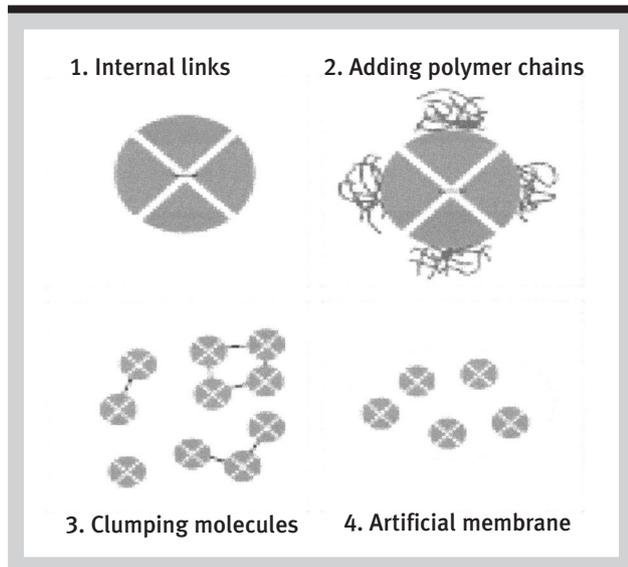
There are a number of HBOC products under investigation that have reached advanced clinical testing. The HBOCs have plasma half-lives in the range of 18 to 58 hours. There are considerable differences among the products listed in Table 3 related to oxygen affinity, molecular stability, the percentage of attendant methemoglobin, side effects, clinical behavior, and hemoglobin concentrations.

Examples of HBOC products being researched are PEG-hemoglobin and pyridoxyl hemoglobin polyoxyethylene, or PHP; both hemoglobin substitutes are rich in lysine residues (42 lysine amino groups) that stabilize the hemoglobin tetramer and increase its molecular weight. Addition of the lysine molecules increases the viscosity and oncotic pressure of the solution and reduces the recognition by the immune system. Polymerized hemoglobin products (Hemolink and Hemopure) are hemoglobin substitutes that are cross-linked with glutaraldehyde, resulting in a polymer of hemoglobin tetramers of various molecular weights and structures.⁸ Cross-linked hemoglobin substitutes are products that are linked together by small bridges producing a stabilized hemoglobin tetramer. An example of such a substitute is diaspirin. Diaspirin has been used as a hemodiluent, permitting hemodilution to be extended to much lower hematocrit levels.¹¹

Advantages of HBOCs are that the mechanics of loading and unloading of oxygen and carbon dioxide are well studied and understood.⁸ Patients are protected from acquiring infectious disease from such transfusions as viral and bacterial inactivation can be achieved by sterilization.

The HBOC tetramers rapidly dissociate to dimers, posing a major drawback because they are not filtered rapidly by the kidneys, which may lead to renal tubular damage. Another disadvantage of HBOCs is their vasoactive property, which can cause hypertension in animals and humans. The hypertension is due to the molecule scavenging and binding to nitric oxide and to stimulation of the adrenergic system, resulting in a vasoactive effect.⁴ Many modifications have been done to alter the hemoglobin molecule so that it remains within the blood vessel, thus reducing or eliminating its ability to scavenge nitric oxide (Figure 2 shows such modifications to the hemoglobin mole-

Figure 2. Methods of modifying the hemoglobin molecule so that the molecule remains in the blood vessel, decreasing the scavenging ability for nitrous oxide



cule). Other disadvantages are the potential for renal toxicity, sepsis, and esophageal dysfunction and abdominal discomfort due to the HBOC binding to endogenous vasoconstrictors (Table 3).

Case study

A recent case report described a 62-year-old patient who underwent abdominal aneurysm repair who received a form of ultrapurified polymerized bovine hemoglobin solution, HBOC-201.¹¹ His hemoglobin concentration before surgery was 13.9 g/dL, and his hematocrit was 40.4%. Estimated blood loss for the total surgery was 8.0 L; his lowest hemoglobin obtained during surgery was 9.5 g/dL, and the lowest hematocrit was 28%. The patient received a total of 2.5 L of salvaged blood, 2 autologous fresh-frozen plasma units, 1 L hydroxyethyl starch, 11.5 L of crystalloid, and 0.89 L of HBOC-201. After surgery, his hemoglobin was 12.5 g/dL, and his hematocrit was 27%.

This product was effective in this patient who lost a large amount of blood and yet did not receive any banked red blood cells. The patient fully recovered without receiving autologous or allogeneic red blood cells.¹¹ The patient's hemoglobin 5 days after surgery was 8.3 g/dL, and his hematocrit was 25%, revealing HBOC-201's short half-life. HBOC-201 is a temporary bridge but has great potential for further investigation and research to extend its half-life.¹²

Hemolink

Hemolink is an HBOC product that is currently on

hold in different medical centers across the country because of adverse reactions. Hemolink is prepared from outdated human red blood cells (Figure 3). The hemoglobin is extracted from the red blood cells, eliminating the blood type antigens, and purified to isolate the hemoglobin subtype A₀. One study included patients undergoing coronary artery bypass graft. Screening and randomized selection was done to determine whether the patient would qualify for the study. Intraoperative and intensive care transfusion triggers were a hemoglobin concentration between 6.5 and 7.5 g/dL or a mixed venous oxygen saturation less than 65%. Another Hemolink transfusion trigger is a decrease in blood pressure not responding to fluid or pharmacological agents. A number of candidates have received the product with a target population of 180 participants (personal communication, B. Spiess, October 2003).

Conclusion

The risks associated with blood transfusions are well known. Hemoglobin substitutes and PFC emulsion are a step closer to being clinically available. More studies and clinical trials are needed before a safe and effective blood substitute becomes available. A good blood substitute has many therapeutic uses. A safe blood substitute can be investigated for extensive use in surgery and potential uses in different medical conditions such as sickle cell anemia and organ ischemia.

Many questions remain unanswered and need to be further investigated and researched to obtain a product free of complications. Patients' outcomes after

Figure 3. Hemolink, a hemoglobin-based oxygen carrier undergoing clinical trials



receiving blood substitutes compared with those of patients receiving conventional, allogeneic blood need to be compared with attention to the immediate and long-term effects. Such research has the potential to produce a product that will save many lives.

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