Artificial Blood: An Update on Current Red Cell and Platelet Substitutes

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INTRODUCTION

The complexity of blood is far too great to allow for absolute duplication in a laboratory. Instead, researchers have focused their efforts on creating artificial substitutes for two important functions of blood: oxygen transport by red blood cells and hemostasis by platelets.

A number of driving forces have led to the development of artificial blood substitutes. One major force is the military, which requires a large volume of blood products that can be easily stored and readily shipped to the site of casualties. Another force is HIV; with the advent of this virus, the medical community and the public suddenly became aware of the significance of transfusion-transmitted diseases and became concerned about the safety of the national blood supply. A third force is the growing shortage of blood donors. Approximately 60% of the population is eligible to donate blood, but fewer than 5% are regular blood donors. The number of units transfused each year has been increasing at twice the rate of donor collection.

Artificial blood products offer many important benefits. First, they are readily available and have a long shelf life, allowing them to be stocked in emergency rooms and ambulances and easily shipped to areas of need. Second, they can undergo filtration and pasteurization processes to virtually eliminate microbial contamination. No product can claim to be 100% risk-free for infectious agents, but these substitutes have a greatly increased level of safety. Third, they do not require blood typing, so they can be infused immediately and for all patient blood types. Fourth, they do not appear to cause immunosuppression in the recipient.

RED CELL SUBSTITUTES

Two major types of red cell substitutes are under development: hemoglobin based and per fluorocarbon (PFC) based. PFCs are completely synthetic hydrocarbon-based compounds. The hemoglobin-based substitutes use hemoglobin from several different sources: human, animal, and recombinant. Human hemoglobin is obtained from donated blood that has reached its expiration date and from the small amount of red cells collected as a by-product during plasma donation. One unit of hemoglobin solution can be produced for every 2 units of discarded blood. There is a concern that the worsening shortage of blood donors will eventually limit the availability of human hemoglobin for processing. The companies that use human hemoglobin are confident in their supply, especially from the plasma centers that use paid donors.

Animal hemoglobin is obtained from cows. This source creates some apprehension regarding the possible transmission of animal pathogens, specifically bovine spongiform encephalopathy. The Biopure Corporation, which uses bovine hemoglobin, has an affiliation with a local breeding farm, allowing close monitoring of the health and diet of the animals. The company is very confident about the safety of its product. Forty units of hemoglobin solution can be obtained per slaughtered cow.

Recombinant hemoglobin is obtained by inserting the gene for human hemoglobin into bacteria and then isolating the hemoglobin from the culture. This process allows for the manipulation of the gene itself to create variant forms of hemoglobin. One unit of hemoglobin solution can be produced from 750 L of Escherichia coli culture.

Once obtained from any of these sources, the hemoglobin must be purified and modified to decrease its toxicity and increase its effectiveness. This task has not proven to be very easy. Research with hemoglobin-based substitutes has actually been under way for over a century. In the 1930s, scientists collected free hemoglobin by lysing red blood cells and then transfused the unmodified product into animals after their blood had been drained. Short-term survival rates were good, but the animals eventually experienced renal failure, intravascular coagulopathy, and vasoconstriction. Much of the toxicity was later attributed to the presence of residual red cell stroma in the product. Hemoglobin also has been determined to have a strong affinity for a relaxing factor derived from endothelial cells (i.e., nitric oxide). By binding to nitric oxide, the free hemoglobin produces unopposed vasoconstriction with subsequent hypertension and bradycardia.

Hemoglobin normally circulates within red blood cells as a tetramer. When free hemoglobin is transfused, the tetramers rapidly break down into dimers and monomers. These small molecules then freely diffuse into the renal tubules and the sub-endothelium. To decrease the toxicity of hemoglobin solutions, manufacturers have had to develop methods to stabilize the hemoglobin tetrameric structure and increase its size. Several such methods now exist: larger molecules are added to the surface, the dimers are cross-linked with sugar molecules, or polymers of several tetramers are formed. Additional modifications of the hemoglobin, such as pyridoxylation, will create a product with near-normal oxygen-binding affinity.

Surface-modified hemoglobin

Surface-modified hemoglobin is created by attaching large molecules, such as polyethylene glycol, to surface lysine groups. This modification also increases the viscosity and oncotic pressure of the solution. Two companies, Enzon and Apex Bioscience, have developed surface-modified hemoglobin solutions. Both of the companies' products, however, triggered moderate vasoconstriction after infusion. The companies have now positioned their products for specialized markets. Enzon is targeting its polyethylene glycol–conjugated hemoglobin product for treatment of patients with stroke and cancer; the small size of the hemoglobin molecules allows them to pass through constrictions and oxygenate areas that cannot be reached by red blood cells. For patients with cancer, the solution can deliver oxygen to tumor cells to increase susceptibility to radiation or chemotherapy. Apex Bioscience is developing its product for treatment of hypotension induced by septic shock.

Cross-linked hemoglobin

To produce cross-linked hemoglobin, small bridges of sugar molecules are covalently attached to the dimers to create a stable tetramer. The US Army had partnered with Baxter Corporation to develop a cross-linked product, HemAssist. However, after increased mortality was noted in phase III trials, product development was discontinued. Baxter had also partnered with Somatogen to produce Optro, a recombinant product produced by *E. coli.* This product is also no longer under development.

Polymerized hemoglobin

To polymerize hemoglobin, surface amino acid groups are linked by reagents such as glutaraldehyde. Polymerized hemoglobin is the only product to date that has not triggered significant vasoconstriction after infusion. Three companies currently have products in phase III clinical trials.

Hemolink, which is being developed by a Canadian company, Hemosol, is created from human hemoglobin polymerized with oraffinose. The solution has a 12- to 18-month shelf life and a 24-hour half-life. PolyHeme is produced by Northfield Laboratories, another Canadian company. It is created from human hemoglobin and has a 1-year shelf life and a 24-hour half-life. During phase III trials, trauma patients received up to 10 units of PolyHeme with minimal side effects. Presently, Northfield can produce about 10,000 units of PolyHeme per year.

Hemopure, produced by Biopure, is a bovine-based hemoglobin. It has a 3-year shelf life and a 24-to 36-hour half-life. Biopure can currently produce about 100,000 units of Hemopure per year; plans to open another plant will allow production to increase to 900,000 units per year. Hemopure is the only hemoglobin solution that has received FDA approval for use in dogs. A recent blinded multicenter trial in patients undergoing infra-renal aortic reconstruction showed that 27% of patients receiving Hemopure were able to avoid transfusion of allogeneic blood. This product has also been transfused several times on a compassionate-use basis. Minimal toxic side effects have been noted.

Perfluorocarbon-based substitutes

PFC-based solutions have been in development for several decades. An article by Clark and Gollan in the 1960s contained the famous photo of a mouse submerged in a container and "breathing" liquid. The liquid was an oxygen-saturated PFC solution. PFCs are synthetic hydrocarbons with halide substitutions and are about 1/100th the size of a red blood cell. These solutions have the capacity to dissolve up to 50 times more oxygen than plasma. Because PFC solutions are modified hydrocarbons, however, they do not mix well with blood and must be emulsified with lipids or oils. Moreover, the best results are obtained if the patient is breathing 100% oxygen at the time of infusion (PaO2 ≥350 mm Hg). The PFCs are inert products. After infusion, the molecules vaporize and are then exhaled over several days.

After halting development of its hemoglobin-based substitutes, Baxter Corporation joined with Alliance Pharmaceutical Corporation to create a new company, PFC Therapeutics, which will market Alliance's Oxygent product. Oxygent has a 2-year shelf life and a 12- to 48-hour half-life. The product is currently in phase III clinical trials for use in cardiac and general surgical patients. In addition, the company has patented a procedure for use of its product in augmented acute normovolemic hemodilution: before surgery, approximately one third of the patient's red cells are removed and stored, and Oxygent and saline are infused to maintain normovolemia and adequate oxygenation during surgery. The stored blood is then infused during or at the end of the surgical procedure. Because the blood lost by patients during surgery is of a lower hematocrit, they lose less of their red cell mass. Some patients have been able to completely avoid transfusion of allergenic blood with this procedure.

Other clinical uses being investigated for PFC solutions in general include replacing red blood cells during acute blood loss, increasing oxygenation of localized areas of hypoxia, increasing oxygenation of solid tumors to improve radio sensitivity, removing gas micro emboli during cardiopulmonary bypass, preserving organs used for transplantation, and allowing liquid breathing for treatment of respiratory distress in premature infants. In addition, PFC-based substitutes would be acceptable to Jehovah's Witnesses, who refuse all human and animal forms of hemoglobin.

Adverse reactions and limitations in the use of oxygen-carrying solutions

Adverse reactions associated with hemoglobin-based products include elevations in blood pressure, gastrointestinal dysmotility, and mild, temporary increases in pancreatic enzymes. Patients also develop jaundice due to the infusion of free hemoglobin. Treatment with PFC-based products can cause mild thrombocytopenia (10% to 15% decrease) and a flulike syndrome. Because patients need to be on high concentrations of oxygen when PFCs

are used, the risk of oxygen toxicity exists with prolonged administration. Since both types of products are taken up by human macrophages, there is also the theoretical risk that macrophage function will be altered.

All current red cell substitutes have a short duration of action—lasting only about 24 hours in the circulation and are very expensive, with estimates at $500 per unit in US. Finally, use of these products can interfere with clinical laboratory testing. Hemoglobin solutions will make the patient's blood specimens appear hemolyzed, and PFC solutions can produce lipemia. Both factors can affect the results obtained by some test systems. Close communication between the clinicians using the products and the laboratory will have to occur if reliable test results are to be reported.

PLATELET SUBSTITUTES

The greatest progress in the field of blood substitutes has been with the oxygen-carrying solutions. However, research on platelet substitutes has been under way since the 1950s. One of the biggest factors pushing the need for platelet alternatives is the 5-day shelf life of the current blood product. This rapid outdate adds additional constraints to an already limited supply. The platelets are also stored at room temperature, thus increasing the risk of bacterial overgrowth. The risk of bacterial contamination of random donor platelets has been estimated to be 1:1500. Ideally, a platelet substitute would have the following properties: effective hemostasis with a significant duration of action, no associated thrombogenicity, no immunogenicity, sterility, long shelf life with simple storage requirements, and easy preparation and administration. Several different forms of platelet substitute are now under development: infusible platelet membranes (IPM), thrombospheres, and lyophilized human platelets. Only one product, IPM, is currently in clinical trials in the USA.

Infusible platelet membranes

Infusible platelet membranes are produced from outdated human platelets. The source platelets are fragmented, virally inactivated, and lyophilized; they can then be stored up to 2 years. Although the platelet membranes still express some blood group and platelet antigens, they appear to be resistant to immune destruction.

One company, Cypress Bioscience Incorporated, manufactures an IPM product that is currently in phase II trials. The company is focusing its product for use in patients who have become refractory to platelet transfusions because of the formation of antibodies to HLA antigen or platelet antigens. The product has successfully stopped bleeding in about 60% of such patients. Overall, the product appears to be safe. No adverse effects have been noted, and there is no evidence that those who receive this product have an increased risk of thrombosis.

Thrombospheres

Thrombospheres (Hemosphere, Irvine, Calif) are not platelets; they are composed of cross-linked human albumin with human fibrinogen bound to the surface.

The mechanism of action has not yet been elucidated. Experimentally, the thrombospheres appear to enhance platelet aggregation but do not themselves activate platelets. Thus far there has been no evidence of thrombogenicity. A similar product, Synthocytes (Andaris Group Ltd, Nottingham, UK), has just entered into clinical trials in Europe.

Lyophilized human platelets

A lyophilized platelet product has been under development since the late 1950s. The current process involves briefly fixing human platelets in para formaldehyde prior to freeze-drying in an albumin solution. The fixation step kills microbial organisms, and the freeze-drying greatly increases the shelf life. The adhesive properties of the platelets appear to be maintained. This product is currently in animal trials.

BLOOD CONSERVATION

Once available, artificial blood substitutes will allow for rapid treatment of anemic patients. Unfortunately, the effects thus far are short lived, so many patients will eventually require allogeneic blood transfusions. With the ongoing shortage of blood donors, which is worsening each year, it has become increasingly important to learn and practice blood conservation measures. For example, in the past, a minimum order for a red cell transfusion consisted of 2 units. Physicians were questioned if they ordered anything less. Now physicians are being educated about transfusing judiciously. It may be possible to maintain the patient by transfusing just 1 unit of blood. That single unit may successfully ameliorate patients' symptoms until their endogenous red cell production increases adequately. Folate, iron, and erythropoietin can also be given to help the bone marrow respond and thus avoid additional transfusions. Limiting laboratory testing and using smaller collection tubes will also conserve patients' blood and prevent worsening of their anemia. Other techniques that enable the practice of "bloodless" medicine and surgery are available but too numerous to detail in this discussion.

With progress, red cell and platelet substitutes may be able to diminish our dependency on donor blood. Until then, it will be exciting to explore the possibilities of the current products once they reach the market.

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