

occurred in the U.S. over the last 25 years, as long as local stocks are repleted appropriately. International incidents have the same scale. The U.S. sent 160 units of RBCs to Kenya and Tanzania to help with the care of the victims of the embassy bombings and to replenish local supplies, but they arrived more than 24 hours after the incident. Local supply was most important in these time-critical situations.

Robust, standardized local and regional blood banking systems provide reservoirs of trained workers and well-equipped staging facilities. Combined with thoughtful, flexible, evidence-based emergency and contingency planning, such systems are more than able to supply blood needs in major wars and disasters. Local availability of high-quality liquid RBCs remains the critical component of blood support for emergencies.

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Synthetic Blood: Myth or Reality?

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Learning Objectives: 1) To describe the current status of blood use and availability in the United States, 2) to identify oxygen-carrying solutions possible to use as alternatives to blood, 3) to review clinical applications of hemoglobin-based O₂ carriers, and 4) to support a hypothesis that hemoglobin based oxygen-carrying solutions could replace a two-unit packed red blood cell infusion for orthopaedic trauma.

Abstract

Synthetic blood may be the solution to a predicted shortfall of packed red blood cells that will be required because of an aging population, increased blood use, and decreased allogeneic collection. With the cost of blood escalating, and blood donation and demand converging, an alternative blood supply must be planned. Two-unit packed red blood cell transfusions account for nearly one-third of red cell use in trauma; approximately 60% of blood is given within the first 24 hours of admission after injury. Oxygen-carrying solutions could be used to avoid the two-unit transfusion for acute blood loss in trauma, for field resuscitation, and when blood is unavailable or refused. More clinical trials are needed for hemoglobin-based O₂ carriers, which provide benefits for trauma patients of no crossmatch, prolonged room temperature storage, volume expansion, stimulation of erythropoiesis, improved rheology in ischemia, and facilitated oxygen diffusion.

Blood Use in the United States

At the Shock Trauma Center in Baltimore in the year 2000, 5,632 trauma patients were admitted, of whom 9.1% (514) received 5,311 units of blood. Figure 1 shows that 72% of the total units of blood (designated by the bar along the top of the graph) were administered to 144 severely injured patients who received more than 10 units. These patients had a mean Injury Severity Score of 32 (30 lived; 35 died) and 38% mortality. Among the remaining 370 patients who received blood, the most frequent mode of infusion was a two-unit red cell transfusion. The 1998 blood transfusion data from Ben Taub Hospital, another major trauma center in Houston, Texas, shows a similar picture. The most frequent mode of

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blood transfusion at Ben Taub Hospital was a two-unit red cell transfusion, with 18.7% of nearly 3,000 trauma patients transfused, twice the transfusion rate of the Shock Trauma Center (J. Hess, personal communication, 2002). Data showing blood use in the military in Vietnam (Fig. 2) shows data that among 2,774 casualties who received nearly 20,000 units of blood, the most frequent mode of blood transfusion was the two- to five-unit administration.²

In 1997, in the United States, the gross domestic supply was just over 12.6 million units of blood; 11.5 million units were administered and 9 million platelets and 3.3 million units of plasma were used.³ A leading question that needs answering is whether this supply is enough. The total allogeneic collection of blood in the United States has fallen from 13.2 million units in 1989 to only 12 million units in 1997. When the 1994 data are compared with the 1997 data at a transfusion rate of 43 units/1,000 population, there was an excess collection over blood administration of 1.3 million units in 1994, but at the same transfusion rate in 1997, there was only a 5.4% margin of excess collection over blood utilization. A shortfall of 4 million units of packed red blood cells (PRBC) is predicted by 2030 because of the aging of the population, increased blood use, and decrease allogeneic collection.⁴ The cost of blood is escalating and blood donation and demand for blood are on a converging pathway. Synthetic blood is positioned to provide a vitally needed alternative supply.

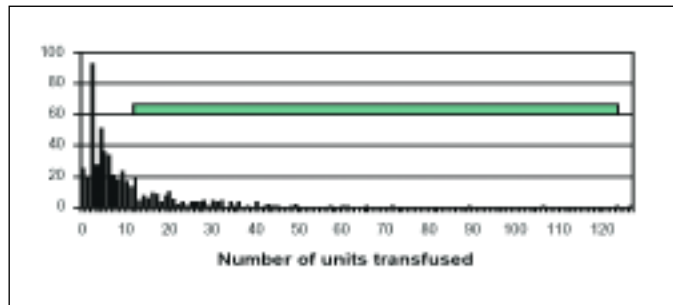


Figure 1. Frequency of number of units of blood transfused at University of Maryland Trauma Center.

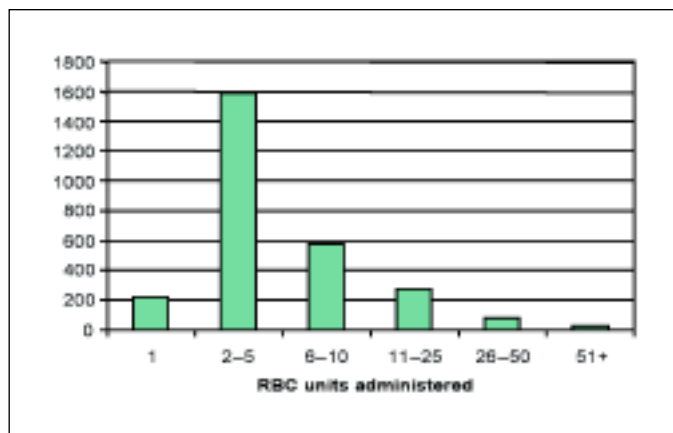


Figure 2. PRBC use among 2,774 Vietnam casualties receiving 19,721 PRBC units of blood. (Adapted from Mendelson.²)

Alternatives to Blood

Red cells can be stored in liquid form for up to 84 days using recently suggested additives.⁵ Red cells can be frozen after addition of glycerol to prevent lysis or they can be freeze-dried or lyophilized; both these methods of storage result in products that take time and resources to reconstitute. Oxygen-carrying solutions may consist of hemoglobin and nonhemoglobin-containing solutions. Stroma-free hemoglobin solutions (from which the stroma or cell wall has been removed) can be modified by polymerization, conjugation, cross-linkage, or liposome encapsulation. The solution can be produced from human, bovine, or recombinant human hemoglobin. Recombinant hemoglobin, which is very expensive to produce, has the same toxicities as other free hemoglobin solutions. Nonhemoglobin-based oxygen-carrying solutions (e.g., perfluorocarbons) are Teflon-like chemicals that carry oxygen proportional to the inspired O_2 that is breathed. Persistent toxicity problems, probably related to complement activation, limits the dose that can be used to the equivalent of about half a unit of red cells.⁶ Dodecafluoropentane (DDFP) has been recently described and shows promise as an extremely low-volume oxygen-carrying resuscitation fluid.⁷ It is low volume because DDFP carries large quantities of oxygen to the tissues as intravascular microbubbles. DDFP picks up oxygen in the lungs and then expands 150 times in volume (because its boiling point is $29^\circ C$), though it still remains smaller than a red cell and it delivers O_2 to the tissues. Recent animal experiments show that 0.3 mL/kg DDFP reverses fatal hemorrhagic shock. One milliliter per kilogram can support the entire O_2 consumption of an adult (300 mL/ O_2 per minute) normally provided by 5 L/min of circulating blood. This artificial O_2 carrier would have immediate battlefield application and civilian trauma application by paramedics in the field or ambulance.

The free hemoglobin solutions are modified to increase their circulatory half-life from 2–3 hours up to 19–28 hours by conjugation or cross-linking. For human-based hemoglobin, pyridoxylation is required to increase P50 from about 12 mm Hg after removal of the cell wall. For bovine products, this is not necessary because O_2 loading and unloading in bovine hemoglobin is chloride-dependent, without need for 2,3-DPG.⁸ Oxygen affinity is not decreased when hemoglobin is removed from the red cell and, in addition, bovine hemoglobin has a pronounced Bohr effect, so P50 of bovine hemoglobin is 43 mm Hg versus 12 mm Hg for human hemoglobin.

Clinical Applications for Hemoglobin-Based O_2 Carriers

The possible applications for hemoglobin-based O_2 carriers (HBOCs) include:

- A true alternative to the oxygen-carrying capabilities of red cells, e.g., elective surgery. *End point* reduced PRBC requirements. Noninferiority to red blood cells (RBCs).
- Resuscitation fluid for prehospital use/alternative to Group O Rh-ve uncrossmatched PRBC. *End point* reversal of ischemia. Noninferiority to crystalloids/colloids alone.
- RBCs unavailable/contraindicated/or refused. *End point* morbidity, mortality compared with matched historical controls (e.g., Jehovah's Witnesses).

- Other applications, e.g., increase sensitivity to radiation, prevent transfusion-related acute lung injury.
End point improved mortality/morbidity over existing treatments.

At a workshop at the National Institutes of Health in 1999, the two summary tables (Tables 1 and 2) were presented by a Food and Drug Administration (FDA) expert.⁹ Preclinical experiences (Table 1) with a large variety of different modified hemoglobins showed the following could occur: vasoconstriction and hypertension, macrophage activation, platelet and red cell aggregation, methemoglobin formation, and evidence for endotoxin release and free radical injury. The clinical experience (Table 2) was vasoreactivity, gastrointestinal upset, and flu-like symptoms. Excess mortality occurred in two clinical trials of the use of HBOCs in ischemic stroke and as a resuscitation fluid for hemorrhaging trauma patients.

Table 1. Summary of Preclinical Experience with Modified Hemoglobins
 (Courtesy of Abdu Alayash, PhD)

- Nitric oxide binding leading to vasoconstriction hypertension, and platelet adhesion
- Macrophage activation leading to cytokine release, vasculitis, and thrombosis
- Platelet and red cell aggregation
- Rapid oxidation to nonoxygen-carrying methemoglobin
- Cellular damage markers of free radical injury
- Enhancement of endotoxin effects

Table 2. Summary of Clinical Experience with Some Modified Hemoglobins
 (Courtesy of Abdu Alayash, PhD)

- Gastrointestinal distress
- Excessive mortality in patients with acute ischemic stroke
- Excessive mortality in resuscitating hemorrhaging trauma patients

There are only two HBOC products that have undergone Phase III FDA human trials: one from Northfield Laboratory (Evanston, Ill.) called Polyheme, produced from human hemoglobin, and the other produced by Biopure Corporation (Cambridge, Mass.) called Hemopure (HBOC-201). HBOC-201 is produced by lysis of bovine red cells from a managed herd of disease-free cattle. HBOC-201 is ultrapurified to remove stroma, undergoes diafiltration to remove potential prions, then is glutaraldehyde polymerized to prolong half-life up to 19 hours. The Northfield product uses human hemoglobin and requires, in addition to glutaraldehyde polymerization, pyridoxilation to increase P50 to 26 mm Hg. The Biopure product has 13 g/dL versus 10 g/dL for the Northfield product. Perhaps the greatest advantage of the HBOC-201 is its 3-year shelf-life at 1–40°C, whereas the Northfield product has to be refrigerated. Both products are isotonic, can carry oxygen, have a half-life of about 20 hours, and do not need typing or

crossmatching. The Northfield product is undergoing a prehospital trauma trial. The Biopure product, HBOC-201, has completed a Phase III orthopaedic trial that recruited 693 patients,¹⁰ and these data are currently being resubmitted to the FDA in response to questions that the FDA would like to have answered. In addition, prehospital studies are underway.¹¹

Physiologic Advantage of HBOC over PRBC

The advantage of having hemoglobin in the plasma is increased diffusive transport of O₂ in the microcirculation. The HBOC molecule is 1/1,000 the diameter of the red cell and therefore improves rheology in the microcirculation. Because HBOC is in the plasma space, oxygen does not have to cross the red cell membrane. Roughly half the oxygen diffusion resistance to red cell tissue O₂ transfer is in the red cell membrane. So this facilitated diffusion combined with the lower O₂ affinity than red cells means that the cellular O₂ delivery from HBOC is three times that of red cells. There are several studies in different animal models in hemorrhagic shock that show HBOC improved splanchnic perfusion, increased tissue oxygenation of skeletal muscles, and restored pancreatic microcirculation in a rat model of severe acute pancreatitis.^{12,13}

The iron in the hemoglobin-based O₂ carrier increases ferritin and erythropoietin in parallel to plasma levels of HBOC. Sixty grams of HBOC provides the equivalent of one unit of blood within 1 week of administration, stimulating reticulocytosis.¹⁴ HBOC acts as an O₂ transport “bridge” until the patient produces his or her own RBC. Plasma hemoglobin has a half-life of 19 hours and requires maintenance with administration of additional doses of HBOC for up to 5 days. At 5 days, reticulocytosis increases and hematocrit rises.

Table 3. Advantages of HBOC Over Stored Blood

HBOC	PRBC
1. Acellular, so flows to ischemic tissues and the microcirculation	RBCs limits blood flow into microcirculation
2. Storage duration 1 year (Polyheme) to 2 years (Hemopure)	Eight-week storage is limit for liquid PRBC
3. No reconstitution necessary, can be stored at 1-38° (Hemopure) or 2-8° (Polyheme)	Refrigeration always needed for storage of PRBC
4. No crossmatch or blood typing required	Crossmatch and typing are routine for PRBC although emergency use of O Rhesus PRBC occurs
5. Uses outdated human (Polyheme) or bovine (Hemopure) hemoglobin as the supply source	Human blood has limited shelf-life before discarding
6. Can be used to avoid disease transmission or religious objections to PRBC	Jehovah’s Witness patients will not accept PRBC. Infectious complications of PRBC administration

A possible use of HBOC in elective orthopaedics would be to replace the two-unit PRBC transfusion. In combination with other blood-saving techniques, HBOC would allow even greater blood-loss surgeries to be performed without need for allogeneic transfusion. When unanticipated blood loss occurs or when blood is not available because of antibodies or when blood is refused for religious reasons, then HBOC can be useful. Even when blood is available, HBOC may be preferred by some to avoid the incidence of human immunodeficiency virus (HIV) (1:1.9 million), hepatitis B (1:180,000), and hepatitis C (1:1.6 million)¹⁵ and human error (1:34,000) in U.S. blood transfusion.¹⁶ The advantages of HBOC over stored blood are summarized in Table 3.

HBOCs for Elective Surgery. The application of HBOCs as an alternative to RBCs in elective procedures has been tested in prospective, randomized, single-blinded studies in several hundred patients undergoing cardiac, vascular, and noncardiac surgery^{17,18} and in South Africa, where HBOC-201 is approved for human use.¹⁹ Approximately one-third of the patients randomized to HBOC-201 avoided blood transfusion throughout hospitalization with no differences in mortality or adverse event occurrences. The Hemopure study of HBOC-201 in orthopaedic patients was a prospective, randomized, single-blinded Phase III study that enrolled 693 patients. The study was designed to determine if HBOC-201 could eliminate RBC transfusion in 35% of patients. The study found that 208 of the 350 patients (nearly 60%) randomized to HBOC-201 avoided transfusion of allogeneic blood for 6 weeks after surgery. The product had a safety profile that was no different from that of blood and an independent blinded panel found that HBOC-201 was not inferior to RBC in overall medical risk.

A subgroup analysis of this Phase III orthopaedic trial included 62 patients who sustained blunt trauma 2–5 days previously and underwent orthopaedic procedures, most frequently acetabular fracture and spine stabilization procedures.²⁰ Patients were randomized to receive red cells or HBOC-201 after informed consent was obtained and a transfusion decision was made. Entry criteria included blood loss >7 mL/kg within 2 hours, BP <90 mm Hg, heart rate >100 beats/min, and hemoglobin <10.5 g/dL. The physiological changes, adverse events, and outcomes were compared within 24 hours of two units of red cells or 60 g of HBOC-201. Thirty-four patients receiving HBOC-201 had mean 1.4 adverse events and 28 receiving PRBC had mean 1.0 adverse events. Serious adverse events (SAEs) occurred in two patients receiving HBOC-201 and none among patients receiving RBC. The SAEs were hypertension in a patient with a known history of hypertension and respiratory failure requiring mechanical ventilation in a patient with a massive pelvic fracture and retroperitoneal hematoma who developed abdominal compartment syndrome. Neither of these SAEs was temporally related to the administration of HBOC-201. The total hemoglobin was significantly higher in the 34 trauma patients after two units of PRBC. One probable reason is that the dose of hemoglobin in two units of blood is about 120–140 g of hemoglobin, whereas only 60 g of HBOC was given. A second reason is that the half-life of HBOC-201 is 19 hours, so only half the initial dose remains at 24 hours. Nonetheless, in this study group, 60% of patients randomized to HBOC avoided blood throughout their hospitalization.

As with use of other HBOCs, the adverse events include a preponderance of flu-like symptoms in patients randomized to HBOC, including pyrexia, gastrointestinal upset, and mild hypertension with elevations in systolic BP of 7–15 mm Hg compared with PRBC administration. This is thought to result

from binding of nitric oxide and release of adrenergic mediators. Laboratory interferences occurred, making it difficult or impossible to accurately measure bilirubin, alkaline PO₄, lactate, and lactate dehydrogenase because of plasma hemoglobin.²¹ Because of methemoglobin and the dissociation curve of bovine hemoglobin, the pulse oximeter reads about 2–5% lower, i.e., saturation of 93–94%. Lastly, clinicians need to change their thinking to start managing patients by plasma hemoglobin levels, a fall in which indicates the need for redosing with HBOC. Total hemoglobin, not hematocrit, is used for assessment of anemia because hemodilution by the cell-free hemoglobin solutions makes hematocrit not proportionally related to total hemoglobin.

Resuscitation Fluid. The first trial of the alpha-alpha cross-linked diasprin hemoglobin solution (Baxter, Deerfield, Ill.), as a resuscitation fluid for trauma patients, recruited 112 patients.²² It was halted after an interim analysis found that mortality was higher in patients receiving HBOC than in normal saline controls. Mortality both at 1 week and 28 days and the multiorgan dysfunction score were also greater. The study was stopped; among the lessons learned was that there was too much intersubject heterogeneity and that end-points were not adequately identified.

A study with Polyheme, the other Phase III product still in clinical trials, showed that infusion of up to six units was safe with no toxicity. Polyheme decreased the number of PRBC needed, but the product was used only in the operating room and very little data were provided about these patients or their long-term outcomes.²³ A second study with Polyheme demonstrated that among 171 patients with urgent blood loss who received Polyheme, 84 received 250 g, equivalent to four to five units of PRBC, and 34 received 500–1,000 g, equivalent to 10 to 20 units of PRBC. This is equivalent to massive transfusion of one to two total blood volume exchanges. In 12 of these patients, red cell hemoglobin fell to less than 1 g/dL; in other words, Polyheme plasma Hb sustained life in 9 of the 12 who survived. A red cell Hb <2 g/dL is incompatible with life, so this study showed the efficacy of sustaining O₂ transport and cellular function independent of red cells. This is illustrated by Figure 3, showing data from 40 patients with RBC hemoglobin <3 g/dL. Plasma hemoglobin (shown as the open part of the histogram) maintained O₂ transport as red cell hemoglobin (shown as the dark part of the histogram) declined below the life-threatening line, representing 3 g/dL of red cell hemoglobin.²⁴

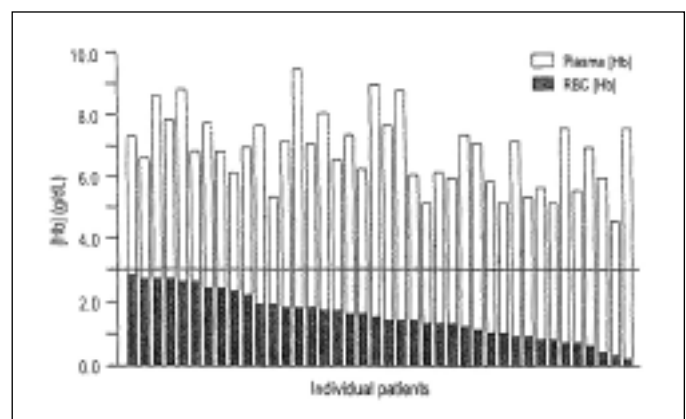


Figure 3. Patient data during hemodilution with Polyheme. (From Gould et al.²⁴ with permission.)

Blood That Is Unavailable, Contraindicated, or Refused. The third indication is when blood is unavailable, contraindicated, or refused. The Jehovah's Witness population is an example of the latter. For the control group in the resuscitation study with Polyheme,²⁰ Gould et al²⁴ used 300 Jehovah's Witness patients who refused blood. The overall mortality was 16% when hemoglobin fell below 8 g/dL. Mortality rose to 64.5% when Hb fell below 3 g/dL and to 100% below Hb 2 g/dL. When the logistical regression showing mortality in patients when receiving Polyheme was plotted against the Jehovah's Witnesses control, the curves of mortality began to separate at 7.3 g/dL and were significantly different when red cell Hb fell below 5.3 g/dL (Fig. 4). Polyheme maintained total Hb (that is red cell and plasma Hb) in excess of 8.5 g/dL throughout. The increased O₂ transport accounted for the reduced mortality with Polyheme.

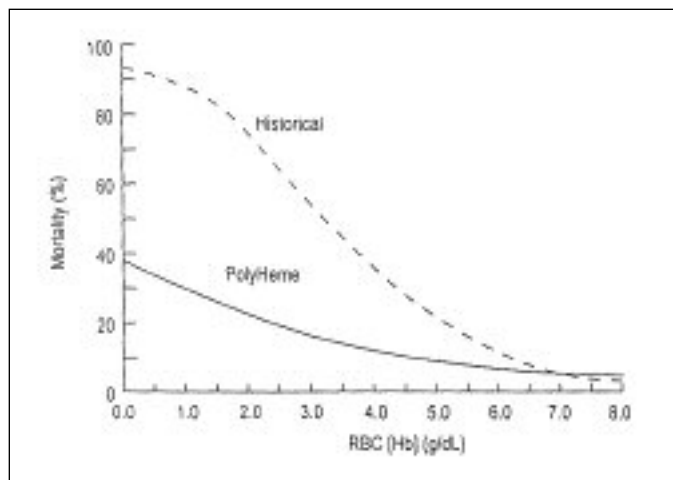


Figure 4. Mortality in patients who received Polyheme compared with historical controls who refused transfusion. Outcome is significantly different ($P < 0.05$) at RBC hemoglobin below 5.3 g/dL. (From Gould et al,²⁴ with permission.)

From the logistical point of view, HBOC will allow avoidance of allogeneic blood exposure. From our data, 60 g HBOC-201 has a similar safety profile to RBC. If one-quarter of the 250,000 U.S. trauma patients requiring blood had HBOC instead, this could save 100,000 units of red cells per year, reduce allogeneic blood exposure by 20–25%, and reduce the number of patients transfused each year by 6–10% nationwide. HBOC use would, therefore, reduce national blood requirements for trauma patients' orthopaedic surgery and provide one potential solution to the predicted future 4 million unit shortfall in blood by 2030. HBOCs can sustain life at red cell Hb <1 g/dL and reduce mortality when red cell Hb falls below 5.3 g/dL.

Conclusion

Two-unit PRBC transfusions account for about one-third of red cell use in trauma and 60% of blood is given within the first 24 hours of admission after injury. Oxygen-carrying solutions could be used to avoid the two-unit transfusion for acute blood loss in trauma, for field resuscitation, and when blood is unavailable or refused. Only two HBOCs continue in clinical trials. Specific benefits of HBOCs used in trauma patients include no crossmatch, prolonged room temperature storage,

volume expansion, stimulation of erythropoiesis, improved rheology in ischemia, and facilitated oxygen diffusion.²⁵

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