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Synthetic blood: Myth or reality?

1) To describe the current status of blood use and availability in the U.S.
2) To identify O_2 carrying solutions possible to use as alternatives to blood.
3) To review clinical applications of hemoglobin-based O_2 carriers.
4) To support a hypothesis that hemoglobin based O_2 carrying (HBOC) solutions could replace a 2 unit packed red blood cell infusion for orthopedic trauma.

Blood use in U.S:

At the Shock Trauma Center in Baltimore in the year 2000, 5632 trauma patients were admitted of whom 9.1% or 514 received 5,311 units of blood. Fig. 1 shows that 72% of the total units of blood (designated by the bar along the top of the graph) were administered to 144 patients who received more than 10 units and were severely injured (1). These patients had a mean injury severity score of 32 and 38% mortality: Among the remaining 370 patients who received blood, the most frequent mode of infusion was a 2 unit red cell transfusion. The 1998 blood transfusion data from BenTaub, another major trauma center in Houston, Texas shows a similar picture. The most frequent mode of blood transfusion at Ben Taub was a 2-unit red cell transfusion, with 18.7% of nearly 3,000 trauma patients transfused, twice the transfusion rate of the Shock Trauma Center (2). 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 2-5 unit administration (3).

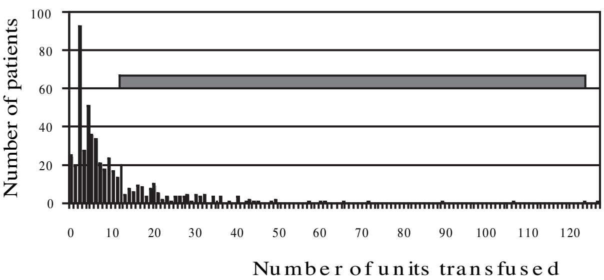

Figure 1. Frequency of Number of Units Transfused at University of Maryland Trauma Center. Of 5632 patients admitted to the University of Maryland trauma center in 2000, 514 (9.1%) received 5311 unit PRBC. 3772 PRBC units (72% of the total) were given to the 144 patients who received more than 10 units PRBC (Data supplied by John Hess, MD). These massively transfused patients had a mean ISS of 32 (lived, ISS=30; died, ISS=35) and a 38% mortality rate.

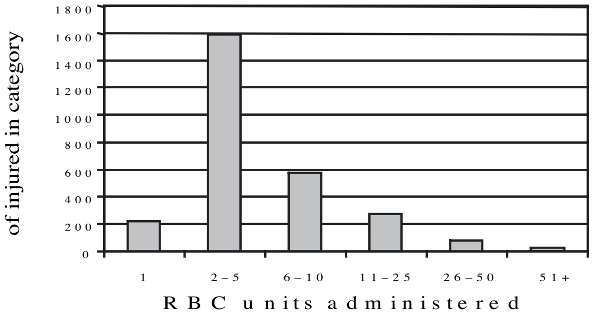

Figure 2. PRBC Use Among 2774 Vietnam Casualties Receiving 19,721 PRBC Units. Adapted from Mendelson J. J Trauma. 1975;15:1-13.

In the US, using the most recent 1997 data available, just over 12.6 million units of blood are administered and 9 million platelets and 3.3. million units of plasma are used (4). A leading question that needs answering is whether this supply is enough. The total allogeneic collection of blood in the USA 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/1000 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 PRBC is predicted by 2030 due to the aging of the population, increased blood use and decrease allogeneic collection. 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.

Alternative 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 free hemoglobin solutions from which the stroma or cell wall has been removed. Liposome encapsulation of hemoglobin is another form of O_2 carrying solution with some platelet toxicity, as is recombinant hemoglobin, which is very expensive to produce and has the same toxicities as other free hemoglobin solutions. Perfluorocarbons are Teflon-like chemicals that carry oxygen proportional to the inspired O_2 that is breathed. The second generation perflubron carries the equivalent of 10g/dl hemoglobin with 50% O_2 inspired concentration. A chemical, Dodecafluropentane (DDFP), has been recently described and shows promise as an extremely low-volume O_2 carrying resuscitation fluid (6). 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 (since its boiling point is 29°C), though it still remains smaller than a red cell and it delivers O_2 to the tissues. Recent animal experiments show that 0.3ml/kg DDFP reverses fatal hemorrhagic shock. 1ml/kg can support the entire O_2 consumption of an adult (300ml/O_2/min) normally provided by 5L/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 mmHg 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 (7). O_2 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 mmHg versus 12 mmHg for human hemoglobin.

Clinical applications for hemoglobin based O_2 carriers

The possible applications for HBOC include:

- **A true alternative to the O_2 carrying capabilities of red cells, e.g. elective surgery.** End point reduced Packed Red Blood Cell (PRBC) requirements. Non-inferority to red blood cell (RBC).
- **Resuscitation fluid for pre-hospital use/alternative to Group O Rh-ve uncross matched PRBC**
  End point reversal of ischemia. Non inferiority to crytalloids/colloids alone.
- **RBC Unavailable/contraindicated/or refused**
  End point morbidity, mortality compared to matched historical controls (e.g. Jehovah Witnesses).
- **Other applications, e.g. increase sensitivity to radiation, prevent transfusion related acute lung injury (TRALI).**
- End point improved mortality/morbidity over existing treatments.

At the NIH Consensus Conference in 2001, the two summary tables (Table 1 and 2) were presented by the Food and Drug Administration (FDA) expert. 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 of vasoreactivity, gastrointestinal (GI) upset and flu like symptoms. Excess mortality occurred in two clinical trials of HBOC's in ischemic stroke and as a resuscitation fluid for hemorrhaging trauma patients.

Table 1. Summary of pre-clinical experience with modified hemoglobins (provided by 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 non-oxygen-carrying methemoglobin
Cellular damage markers of free radical injury
Enhancement of endotoxin effects

Table 2. Summary of clinical experience with some modified hemoglobins (provided by Abdu Alayash, PhD)

Vasoconstriction and hypertension
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 Lab called Polyheme, produced from human hemoglobin, and the other produced by Biopure Corporation 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 ultra purified 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 glutaraldelyde polymerization, pyridoxilation to increase P50 to 26 mmHg. The Biopure product has 13g/dl versus 10g/dl for Northfield. 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 cross matching. The Northfield product is undergoing a pre-hospital trauma trial. The Biopure product HBOC-201 has completed a Phase III orthopedic trial that recruited 693 patients (8) and is also undergoing a pre-hospitla trauma trial. These data are currently being resubmitted to the FDA in response to questions that the FDA would like to have answered.

Physiologic Advantage of HBOC over PRBC

The advantage of having hemoglobin in the plasma is increased diffusive transport of O_2 in the microcirculation. The HBOC molecule is 1/1000th the diameter of the red cell and therefore improves rehology 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 O_2 transfer is in the red cell membrane. So, this facilitated diffusion combined with the lower O_2 affinity than red cells, means that the cellular O_2 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 micro circulation in a rat model of severe acute pancreatitis (9,10) .

The iron in the hemoglobin-based O_2 carrier increases ferritin and erythropoietin in parallel to plasma levels of HBOC. 60g HBOC provides the equivalent of one unit of blood within one week of administration, stimulating reticulocytosis (11). HBOC acts as an O_2 transport "bridge" until the patient produces their own RBC. Plasma hemoglobin has a half life of 19 hours and requires maintenance with administration of additional doses of HBOC for up to five days. At five days reticulocytosis increases and hematocrit rises.

A possible use of HBOC in elective orthopedics would be to replace the 2-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 HIV (1:1.9 million) Hepatitis B (1:180,000) and Hepatitis C (1:1.6 million) (12) and human error (1:34,000) in U.S. blood transfusion (2001 data)

a) HBOC's for Elective Surgery

The application of HBOC's as an alternative to RBC in elective procedures has been tested in prospective randomized single blinded studies in several hundred patients undergoing cardiac, vascular and non-cardiac surgery (13,14) and in South Africa where HBOC-201 is approved for human use (15). About 1/3rd 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 orthopedic patients was a prospective randomized single blinded Phase III Study that enrolled 693 patients. The study was designed to determine

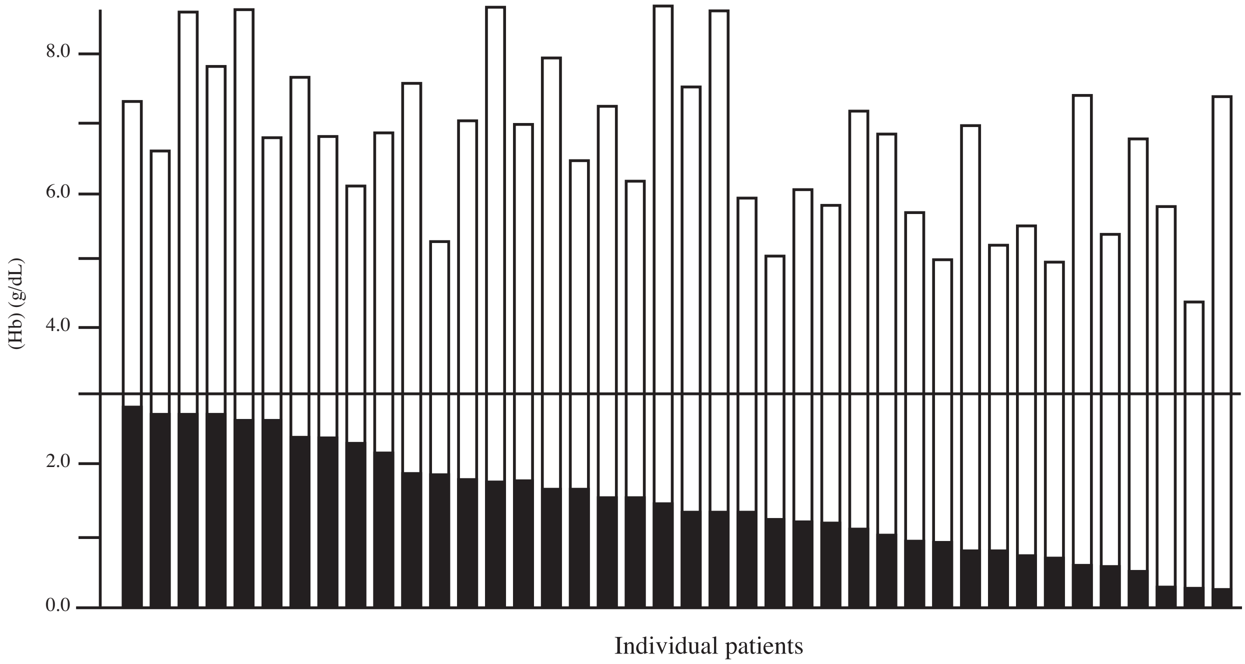

Figure 3. Individual patient data for 40 patients with nadir RBC [Hb) ≤3 g/dL, showing the total [Hb] as the sum of RBC [Hb] (■) and plasma [Hb] (□). Line represents life-threatening level of 3 g/dL, [Hb], hemoglobin concentration. Gould et. al J Am Coll Surg 2002; 195:445-455.

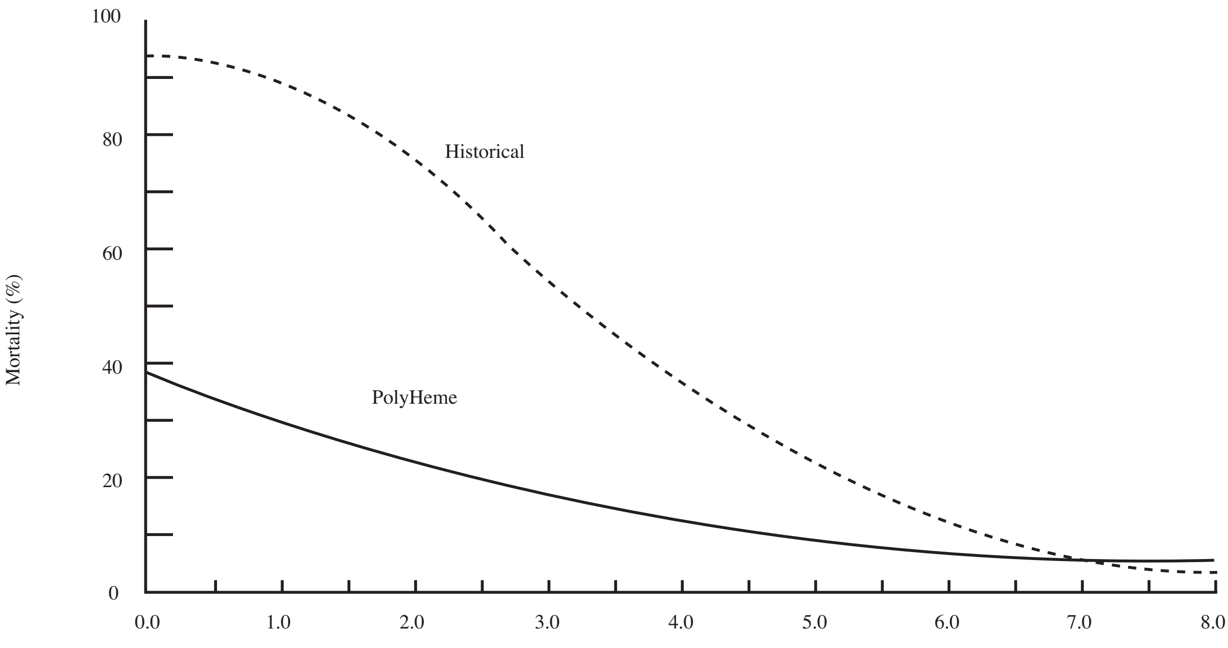

Figure 4. Logistic regression showing mortality in patients who received Polyheme and in historical controls. Mortality increases in both groups as RBC [Hb] falls. Curves begin to separate at RBC [Hb] of 7.3 g/dL and become significantly different (p< 0.05) at RBC [Hb] below 5.3 g/dL. [Hb], hemoglobin concentration. Gould et. al J Am Coll Surg 2002; 195:445-455.

if HBOC-201 could eliminate RBC transfusion in 35% of patients. The study found that 208 of the 350, or nearly 60% of patients randomized to HBOC-201, avoided transfusion of allogeneic blood for six weeks after surgery. The product had a safety profile no different than blood and an independent blinded panel found that HBOC-201 was not inferior to RBC in overall medical risk (8).

A subgroup analysis of the above described Phase III orthopedic trial included 62 patients who sustained blunt trauma 2-5 days previously and underwent orthopedic procedures, most frequently acetabular fracture and spine stabilization procedures (16). 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 mmHg, heart rate > 100 beats/min. and hemoglobin < 10.5 g/dl. The physiological changes adverse events and outcomes were compared within 24 hours of 2 units red cells or 60g 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 (SAE's) occurred in two patients receiving HBOC-201 and none among patients receiving RBC. The SAE's 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 SAE's was temporarially related to the administration of HBOC-201. The total hemoglobin was significantly higher in the 34 trauma patients after 2 units PRBC. One probable reason is that the dose of hemoglobin in 2 units of blood is about 120-140g of hemoglobin, whereas only 60g of HBOC was given. A second reason is that the half life of HBOC-201 is 19 hours, so only half the initial does remain at 24 hours. Nonetheless in this study group 60% of those patients randomized to HBOC avoided blood throughout their hospitalization.

As with use of other HBOC's the adverse events include a preponderance of flu-like symptoms in those patients randomized to HBOC including pyrexia, gastrointestinal upset, mild hypertension with elevations in systolic BP of 7-15 mmHg compared to 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, lactate dehydrogenase because of plasma hemoglobin (17). 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 re-dosing 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.

b) Resuscitation Fluid

The first trial of the alpha alpha cross-linked diasprin hemoglobin solution (Baxter), as a resuscitation fluid for trauma patients, recruited 112 patients (18). It was halted after an interim analysis found that mortality was higher in those patients receiving HBOC than normal saline controls. Mortality both at one week and 28 days and the multi-organ dysfunction score, were also greater. The study was stopped and among the lessons learned was that there was too much inter-subject 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 was provided about these patients or their long-term outcomes (19). A second study with Polyheme demonstrated that among 171 patients with urgent blood loss who received Polyheme, 84 of these patients received 250g equivalent to 4-5 units PRBC, and 34 received 500-1000g equivalent to 10-20 units 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 1g/dl – in other words Polyheme plasma Hb sustained life in the 9/12 who survived. A red cell Hb < 2g/dl is incompatible with life, so this study showed the efficacy of sustaining O_2 transport and cellular function independent of red cells. This is illustrated by Fig. 3 showing data from 40 patients with RBC hemoglobin < 3g/dl. Plasma hemoglobin shown as the open part of the histogram maintained O_2 transport as red cell hemoglobin shown as the dark part of the histogram declined below the life-threatening line representing 3g/dl red cell hemoglobin (20).

c) Blood unavailable, contraindicated or refused

The third indication is when blood is unavailable, contraindicated or refused. The Jehovah Witness is an example of the latter. For the control group in the resuscitation study with Polyheme (10), Gould et al used 300 Jehovah Witness patients who refused blood. The overall mortality was 16% when hemoglobin fell below 8g/dl. Mortality rose to 64.5% when Hb fell below 3g/dl and 100% below Hb 2g/dl. When the logistical regression showing mortality in patients when receiving Polyheme was plotted against the Jehovah Witnesses control – the curves of mortality began to separate at7.3g/dl and were significantly different when red cell Hb fell below 5.3g/dl. (Fig. 4) Polyheme maintained total Hb (that is red cell and plasma Hb) in excess of 8.5g/dl throughout. The increased O_2 transport accounting for the reduced mortality with Polyheme.

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

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