The Status of Hemoglobin-Based Red Cell Substitutes

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Abstract

Red cell substitutes are currently under development for use in a variety of surgery and trauma-related clinical conditions. The need for artificial oxygen-carrying fluids continues to be driven by the shortage of donor blood, the complex logistics of blood banking, the risk of virally transmitted diseases, current transfusion practices, and the projected increased demand for blood products in the future. The effort to develop a replacement for the red cell component has evolved over the last century and has presented a number of significant challenges including safety and efficacy concerns. Recent progress in understanding the fundamental interactions of hemoglobin with the body at the molecular, cellular and tissue levels has led to the production of improved red cell substitutes suitable for clinical testing. Currently, seven products are being tested for a variety of applications including trauma, surgery, sepsis, cancer and anemia. Although some of these trials were unsuccessful, the majority of the available products exert no toxicity or only low level side effects. Encouraging results in early clinical trials with oxygen-carrying fluids support further development of these products and have increased the hope that a usable oxygen-carrying fluid will soon be available in the clinic. The purpose of this review is to provide up-to-date information on the status of these products with special emphasis on pre-clinical and clinical experience.

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Although donor blood transfusion is life saving in many patients with acute massive blood loss, it is plagued by a myriad of clinical, logistic and economic drawbacks that severely limit its use. Therefore, there is an urgent need to develop a safe and effective artificial oxygen-carrying red cell substitute. The leading strategy in the search for such a resuscitation fluid employs the endogenous oxygen-carrying molecule, hemoglobin. This review describes progress towards the development of synthetic hemoglobin-based oxygen-carrying fluids, presents the promise and challenges in their production, and outlines future research directions. Although the author was involved in the development of various red cell substitutes this review aims to be non-biased and balanced.

Rationale for development

Improving oxygen delivery to ischemic tissues is the primary goal in the treatment of hemorrhagic shock. To achieve this aim, it is standard care to transfuse allogenic blood to patients with acute massive blood loss. Unfortunately, the use of donor blood is associated with multiple problems; these problems constitute the principal force driving the development of red blood cell substitutes.

To safely transfuse blood a whole battery of costly and time-consuming compatibility testing is required. Also, in spite of improved screening tests there is a risk of transfusion-transmitted diseases such as human immunodeficiency virus and hepatitis C, currently estimated to be 1:500,000 and 1:30,000, respectively. In addition, bacterial contamination of blood, although uncommon, can cause grave consequences while clerical mistakes or the presence of rare recipient antibodies may induce severe hemolytic and anaphylactic reactions. Allogenic blood transfusion can also lead to a variety of metabolic effects including hyperkalemia, hypocalcemia, and alkalosis. Logistically, blood has a short shelf-life that currently does not exceed 42 days, necessitating refrigerating machinery for storage and transport. In economic terms, the collection, screening, storage and transfusion of donor blood is expensive. Current estimates indicate that between 12 and 13 million units of red blood cells are used each year in the USA alone. Thus, at a cost of U.S. $150–225 per unit, red blood cell transfusion poses an annual financial burden of approximately $2–3 billion. Finally, in time of war or major disaster, the supply of whole blood as well as the infrastructure necessary for its collection, storage and transportation may be disrupted.

Taken together, these factors make the development of artificial oxygen-carrying fluids a priority in critical care medicine. Since these products are antigen free, easily adaptable to scale-up production, have a long shelf-life, and can be easily administered, it is anticipated that synthetic oxygen-carrying fluids will offer significant advantage over human erythrocytes with respect to availability, storage, transportation, sterility and administration. The economic impact of these products, however, is still unknown.

Strategies for development

Free hemoglobin

The hemoglobin tetramer has been sought for many years as the leading candidate for an oxygen-carrying fluid because of its

natural property to carry, deliver and release oxygen in a cooperative manner, its high saturation at ambient oxygen pressures, and its capacity to harbor as much as 1.39 ml oxygen/g protein. In addition, free hemoglobin solutions have no antigenic determinants, which are embedded solely within the erythrocyte membrane, and therefore could potentially eliminate many of the problems encountered with whole blood transfusion.

• *Pre-clinical data*

The pre-clinical description of acellular hemoglobin solutions began with the characterization of the structure and oxygen-carrying kinetics of the hemoglobin molecule itself. As the development of hemoglobin-based red cell substitutes emerged in several laboratories, many of their physicochemical and oxygen-carrying properties were characterized and found to vary significantly [Table 1].

Early on in the development of this product, it became apparent that hemoglobin purified from red blood cells was associated with several fundamental problems. First, the lack of cellular structure caused a loss of 2,3-DPG, which markedly reduced the ability of acellular hemoglobin to unload sufficient amounts of oxygen into tissues [1]. Second, free hemoglobin was found to be very unstable in the plasma where it rapidly dissociates to dimeric forms. This has several important consequences: a) an acute increase in the colloid oncotic pressure of the plasma, which limits the amount of acellular hemoglobin that can be infused without risking volume overload; b) filtration of hemoglobin through the glomerular membrane, which leads to both nephrotoxicity [2] and rapid clearance from the circulation [3]; and c) enhanced extravasation of hemoglobin through vascular endothelium to the vascular smooth muscle layer and induction of vasoconstriction [4]. The mechanism of this vasopressor effect is still obscure. Nevertheless, it is possible that the rapidly diffusing free hemoglobin molecules modulate the production of two potent vasodilators, nitric oxide and carbon monoxide. Modulation may occur by high affinity binding of nitric oxide and by up-regulation of heme oxygenase, which facilitates carbon monoxide production [for review see 5]. Alternatively, it has been proposed that enhanced oxygen delivery by free hemoglobin triggers autoregulatory arteriolar spasm [6].

To avoid these undesired properties, acellular hemoglobin has been chemically modified. Oxygen affinity was increased by pyridoxalation [7], and stability enhanced by polymerization [1], cross-linking of the α chains [8], incorporation of polyethylene glycol [9] or by the recombinant production of α1-α2 hemoglobin [10]. Also, production methods have been improved to eliminate cellular debris and endotoxin contamination, which could account for some toxicities. Nevertheless, in spite of these improvements animal studies with modified hemoglobin yielded mixed results. For example, while some investigators observed no renal toxicity [11,12] other laboratories still demonstrated impaired renal function following infusion of modified acellular hemoglobin preparations [13,14]. Similarly, vasoconstriction was still a side effect in several modified solutions [15,16]. These contrasting reports clearly indicated that finding a replacement for hemoglobin that works outside of its native and protective environment – the red blood cell – has turned out to be a much tougher problem than expected.

• *Clinical data*

Competitiveness in the field resulted in a paucity of peer-reviewed scientific information and a deluge of non-peer-reviewed data of questionable validity. Thus, the ability to verify and critically analyze information regarding clinical trials is very limited and subject to significant inaccuracies. Nevertheless, to provide updated information the authors opted to include data from all available sources with the assumption that the reader is aware of the limitations of such a review.

Early clinical experience with stroma-free hemoglobin

Table 1. Physicochemical and oxygen-carrying properties of hemoglobin-based solutions

Product	Hb (g/dl)	MetHb (%)	P_{50} (mm Hg)	Hill coefficient	$T_{1/2}$ (hr)	COP (mm Hg)	Viscosity (cps)	LPS (EU/ml)
Pyridoxalated PEG-modified Human Hb	8±0.5	<5	20±3	1.5±2.0	9 human	40	3.2	<0.2
α-α cross-linked human Hb	10	4	32	2.7	2–14*	42	1.0	<0.24
PEG-modified Bovine Hb	6	<5	15	NA	44 human	n.a	NA	<0.5
O-Raffinose cross-linked and polymerized human Hb	10	<10	34	1.0	24	26	1.15	0.06
Pyridoxalated, gluteraldehyde polymerized human Hb	10	<3	28–30	NA	24 human	20–25	NA	NA
Glutaraldehyde polymerized bovine Hb	13	<10	38	NA	24 human	17	1.3	<0.5
α-α cross-linked mutant of human Hb (from *E. coli* cells)	5	<5	33	2,4	NA	NA	NA	NA
Liposome-encapsulated bovine Hb	10	12	18	2.8	16 rodent	0.7	NA	?

* Dose dependent.

? Currently under validation due to potential interference of LEH components with the limulus amoebocyte lysate assay. Rabbit pyrogen test is negative.

MetHb = methemoglobin, COP = colloid oncotic pressure, NA = not available.

solutions was less than encouraging. Patients experienced hypertension, nephrotoxicity and coagulopathies [17] that have since been attributed to stromal contaminants. Clinical studies with improved modified hemoglobin preparations also provided conflicting evidence regarding their safety. For example, in 1989 a safety trial with polymerized human hemoglobin in post-operative anemic but stable patients was discontinued due to side effects such as shortness of breath and chest discomfort [18]. Also, in Germany, a carefully prepared solution of modified acellular hemoglobin caused severe renal toxicity in two healthy volunteers. Furthermore, a safety study with modified bovine hemoglobin in the United States, previously reported to exert no side effects in 10 healthy volunteers in Guatemala, was abruptly halted for undisclosed reasons.

Seven companies are currently testing their acellular hemoglobin products in clinical trials [Table 2]. The main distinctions between these products are the source of hemoglobin used (human, bovine, recombinant human), the size of the molecular product (tetramer, polymer), and the agents used to chemically stabilize and decorate the hemoglobin molecule. Additionally, although all products are pyrogen free, sterile and highly purified prior to testing in humans, it should be pointed out that differences in the degree of purification might explain some of the differences observed when these products are administered.

Baxter Healthcare Corporation (Deerfield, IL, USA) was one of the first companies to begin clinical testing of their product, HemAssist™, a diaspirin cross-linked human hemoglobin solution. According to Baxter, two phase I [19,20] and five phase II safety protocols have been completed and demonstrated a hypertensive effect that was not associated with adverse outcome. This property was viewed by Baxter as a potential benefit of HemAssist™ to be exploited in the resuscitation of hypotensive patients. It should be noted however that, to the best of our knowledge, no reports on the phase II trials were published in refereed journals.

Baxter has also initiated phase III clinical trials in both the U.S. and Europe in the early hospital treatment of hemorrhagic trauma and in peri-operative patients. Very recently, Baxter abruptly halted its U.S. trauma study of HemAssist™ after a review of the first 100 participants showed increased mortality (46.2%) in the experimental group compared with the projected 42.6% mortality rate and the 17.4% mortality in the control group. Several months later Baxter also suspended its European tests of HemAssist™ in trauma victims after early results demonstrated no statistical benefits of this product. Based upon these results Baxter has discontinued its support to the HemAssist™ Project as a viable red cell substitute for trauma and emergency surgical applications.

Northfield Labs Incorporation (Evanston, IL, USA) is developing PolyHeme™, a polymerized human hemoglobin solution. In 1997, the company reported a single-center, prospective, non-randomized, open-label study that evaluated the use of PolyHeme™ in patients with acute blood loss due to trauma or surgery [21]. Inclusion criteria were acute bleeding, anticipation of low hemoglobin and/or systolic blood pressure of <100 mm Hg. Thirty-nine patients were included of whom 14 were given 1 unit of PolyHeme™, 2 patients were given 2 units, 15 patients were transfused with 3 units and 8 patients received 6 units. This preliminary study provided encouraging data. First, PolyHeme™ maintained total hemoglobin concentration when red blood cell hemoglobin dropped to 2.9 g/dl. Second, 23 patients avoided allogenic blood transfusion during the first 24 hours. Third, pre- and post-transfusion values of blood pressure, creatinine clearance and liver function tests did not differ statistically. Very recently Northfield reported the first prospective randomized study that directly compared donated blood with a red cell substitute in trauma patients [22]. This double-center open-label study included trauma patients with acute bleeding, systolic blood pressure <100 mm Hg, or patients who were anticipated to drop their hemoglobin. Forty-four patients were randomly assigned into two matching groups to receive up to 6 units of PolyHeme™ or red blood cells. The reported results were favorable: PolyHeme™ maintained hemoglobin levels, had no serious side effects and reduced the number of units of allogenic blood transfused.

Table 2. Clinical trials with modified acellular hemoglobin solutions (In alphabetical order)

Company	Product	Product name	Clinical program	Clinical Phase
Apex	Pyridoxalated PEG-Modified Human Hb (>85 kD)	PHP	• Sepsis	I
Baxter	α-α-cross-linked human Hb (64.5 kD)	HemAssist™	• Surgery • Organ failure • Trauma • Stroke • Hemodynamic maintenance	II/III (USA and Europe) Discontinued
Biopure	Gluteraldehyde Polymerized Bovine Hb (>150 kD)	Hemopure™	• Hemodilution/surgery • Anemias	Ib/II
Enzon	PEG-Modified Bovine Hb (>100 kD)	PEG-Hb	• Cancer adjunct (Radiosensitization)	I
Hemosol	O-Raffinose Cross-linked and Polymerized Human Hb (>100 kD)	HemoLink	• Surgery • Anemias	I Phase II approved
Northfield	Pyridoxalated, glutaraldehyde Polymerized Human Hb (>150 kD)	PolyHeme™	• Surgery • Trauma	II
Somatogen	α-α Cross-linked Mutant of Human Hb (from *E. coli*) (64.5 kD)	Optro™	• Surgery/hemodilution • Trauma	Ib/II

Nevertheless, the study was criticized for its small number of patients, which precluded demonstrating efficacy or harm, and for lacking efficacy data including morbidity and mortality. In spite of this criticism, Northfield remains the only company claiming to have given this much hemoglobin to patients without any reported side effects.

Somatogen Incorporation (Boulder, CO, USA) is the leading company for the development of recombinant hemoglobin [10] and is the only company with recombinant hemoglobin in clinical trial. Its product, Optro™ (formerly rHbl.l), is recombinant human hemoglobin produced by *Escherichia coli* cells. Optro's development has been slow due to the meticulous purification steps required to remove pyrogens inherent from the bacterial production method. Initial phase I clinical trials were discouraging and Optro™ has been reported to cause fever, headache and elevated blood pressure in healthy volunteers. However, a change in the purification process appears to have solved these problems, and in two recent phase IIa trials of the drug in 33 elective surgery patients no significant clinically related side effects have been observed [23,24]. Most importantly, no kidney toxicity was seen at doses between 50 and 100 g of Optro™. In early 1996, Somatogen reported results of its phase II multi-center trial in patients who received Optro™ to restore blood volume during acute normovolemic hemodilution in conjunction with elective surgery. Doses ranged from 25 to 100 g to replace up to 4 units of blood. Some patients exhibited elevated amylase and lipase levels that returned to normal within 12–24 hours post-infusion. No other clinically significant adverse event was reported. Phase II studies with Optro™ are planned in ten U.S. medical centers to test this product in the setting of cardiopulmonary bypass. It should be noted that the recent purchase of Somatogen by Baxter will most likely add momentum to the development of Optro™.

In 1990, Upjohn committed $179 million to Biopure Corporation (Cambridge, MA, USA) for the development of the bovine oxygen carrier, but in 1991 it had to stop a phase I study when the product Hemopure™ caused chest pain in healthy volunteers. However, according to Biopure, that problem has long since been resolved and no notable safety concerns, including immunological reactions to the bovine protein, have been seen in subsequent trials. In a phase I study in radical prostatectomy patients, up to 45 g have been safely administered postoperatively without serious side effect [25]. Data are currently being analyzed for recently completed phase II studies in patients with sickle cell anemia and patients undergoing repair of aortic abdominal aneurysm. In these studies, patients received 60 and 150 mg of Hemopure™. Biopure has also initiated a phase III clinical study to evaluate the safety and effectiveness of Hemopure™ when compared to allogenic red blood cells in elective orthopedic surgery. It should be noted that: a) only Biopure has succeeded in producing a room temperature stable bovine hemoglobin solution, and b) Upjohn no longer supports Biopure.

In phase I clinical trials of HemoLink™, an o-raffinose cross-linked and polymerized human hemoglobin manufactured by Hemosol Incorporation (Etobicoke, Ontario, Canada), 33 healthy volunteers were given up to 600 mg/kg of HemoLink™ with no adverse cardiovascular, pulmonary or renal side effects [23,24]. However, marked gastrointestinal side effects were observed in some subjects receiving more than 500 mg/kg of the product, which according to a company spokesman can be readily and effectively managed by commonly used pharmacological agents. In 1998, Hemosol reported the successful completion of a phase II clinical study with HemoLink™ in orthopedic surgical patients receiving up to 500 ml of this red cell substitute. In the same year, Hemosol announced it had received approval from the Canadian regulatory authorities to initiate a phase II study of HemoLink™ in patients undergoing coronary artery bypass grafting surgery in Canada. In this study, HemoLink™ will be tested for its ability to reduce the need for allogenic blood. Based on the demonstrated effect of HemoLink™ to expand red cell precursors in the laboratory, renal dialysis trials are now in progress in both Canada and the United States to test HemoLink's efficacy in the treatment of anemia. Another modified hemoglobin solution is pegylated bovine hemoglobin (PEG-Hb), produced by Enzon Incorporation (Piscataway, NJ, USA). This product is being developed as an adjunct agent for radiation therapy based upon its demonstrated ability to enhance oxygen delivery to hypoxic tumors, which renders them more susceptible to radiation therapy. Initial phase I safety studies were conducted in 28 healthy subjects in whom up to 44 g of PEG-Hb was administered. In this study, no major organ toxicities were observed, however significant epigastric and abdominal discomfort was noted which resolved within 24 hours without treatment [23,24]. Preliminary results of testing a more highly purified formulation showed an absence of epigastric discomfort and a substantial reduction in the frequency, duration and severity of abdominal symptoms. Enzon has recently begun a phase Ib multiple-infusion, dose-escalation study in which the safety and tolerance of PEG-Hb will be evaluated in 20 patients with hypoxic solid tumors receiving palliative therapy. This study should provide a platform for initiation of a phase II/III clinical program in the future.

Pyridoxalated hemoglobin polyoxyethylene produced by Apex Bioscience Incorporation (Research Triangle Park, NC, USA) is another modified acellular hemoglobin preparation currently being tested in clinical settings. One version of this product is already in clinical trials as a treatment for acute sepsis-induced hypotension. In a preliminary phase I study PHP caused no significant adverse reactions in 12 healthy volunteers, even at the highest dose (8 g) [23,24]. A phase II study is being planned in volume-refractory sepsis patients to see if PHP can replace vasoactive drugs currently given to non-responding sepsis patients in an attempt to elevate their blood pressures.

PEG-Hb = pegylated bovine hemoglobin
PHP = pyridoxalated hemoglobin polyoxyethylene

Phase I/II clinical trials will also be launched to test the ability of PHP to reduce tumor size based upon its selective constrictive effect on tumor blood vessels.

In summary, clinical trials have provided important information regarding the safety of acellular hemoglobin solutions. Currently, only one product has shown promise at high doses, whereas several other red cell substitutes tested at lower doses demonstrated none or only minimal side effects. Thus, although the original aim of complete red cell replacement is still being solicited, there are only limited data to support it. On the other hand, the lack of significant side effects at low doses of most red cell substitutes supports their use to reduce allogenic blood transfusion and to combat a variety of surgery-related diseases such as sepsis and cancer.

Liposome-encapsulated hemoglobin

Another strategy toward the development of an oxygen-carrying fluid has been the encapsulation of hemoglobin within a synthetic phospholipid bilayer. This approach continues to be driven by the difficulties encountered in the development of acellular hemoglobin and the need to shield the hemoglobin molecule from adverse interactions with cells, tissues and organs. The phospholipid bilayer of the liposome offers no barrier to gas exchange, and early physicochemical studies demonstrated oxygen loading and unloading kinetics similar to that of the red blood cell [26]. In addition, liposomal membranes have been shown to effectively maintain the hemoglobin molecule within the intravesicular compartment, and encapsulated hemoglobin as well as other encapsulated materials such as pyridoxal-5-phosphate do not readily leak from the liposome either *in vitro* or *in vivo*.

LEH has been shown to effectively carry oxygen [26] and to prevent hemoglobin dissociation, thereby increasing circulation time as well as eliminating nephrotoxicity. Also, the condensation of hemoglobin into a reduced number of particles diminishes the oncotic activity of LEH compared to acellular hemoglobin and thus enables transfusion of larger doses of the oxygen-carrying molecule.

• *Pre-clinical data*

LEH, whose physicochemical properties were well defined [Table 1], has been extensively tested for its safety, efficacy and immunological effects in a variety of animal models. Side effects of early preparations including hypertension, hypotension, hemoconcentration and leukocytosis [27] were eliminated followed by improved modifications that demonstrated minimal side effects in several species including primates [28–30].

Since LEH is cleared from the circulation primarily by the reticuloendothelial system, a major safety concern in the development of this product was the possibility that massive LEH transfusion could expose patients to sepsis. Thus, the effect of LEH on endotoxin-induced responses was tested in the rat. In this experiment, LEH was shown to exacerbate consequences of endotoxemia only if administered prior to a very high dose of lipopolysaccharide or if the LEH-LPS time interval was shorter than 12 hours [31]. Because the clinical relevance of animal models of endotoxin-induced "sepsis" is debatable, the significance of these results should await further investigation in improved animal models that mimic human sepsis.

In parallel to the safety studies, the efficacy of LEH has been demonstrated in a variety of animal models including a rat model of 50% isovolemic exchange transfusion [32] and severe controlled hemorrhagic shock [33]. In a different set of experiments, LEH has been shown to significantly diminish the vasoconstrictor activity of both non-modified and modified (α-α cross-linked) hemoglobin in pre-contracted rabbit arterial segments [34].

Taken together, the pre-clinical data clearly support further development of LEH, and a decision whether to promote this product towards phase I clinical trials is anticipated shortly.

Future directions

Although the development of hemoglobin-based oxygen-carrying fluids has significantly progressed, there are clearly many unresolved issues concerning their properties and safety as well as their testing in clinical settings.

First, there are no clear definitions regarding some fundamental characteristics of the ideal hemoglobin-based red cell substitute. For example, it is not yet known what the optimal hemoglobin concentration of these fluids should be, which reflects, at least in part, deficient knowledge regarding the ultimate "target" post-resuscitation hemoglobin level. Also, the viscosity required for optimal flow and delivery of the oxygen-carrying particles is still unknown. Finally, the preferred circulation time is still a subject of much debate. On one hand, it could be more beneficial to have a resuscitation fluid that will persist in the circulation for at least 90–120 days to enable newly produced red blood cells to resume the responsibility of oxygen delivery. On the other hand, a much shorter half-life that will support the patient only during the first but critical post-injury days may be advantageous because it may minimize potential chronic toxicities. Thus, comprehensive studies to address these issues should be conducted to expedite and improve the development of oxygen-carrying resuscitation fluids.

Second, one of the major concerns in the development of all hemoglobin-based red cell substitutes is the source of the hemoglobin used. To date, most laboratories are using outdated human or bovine hemoglobin. The first is subject to many of the drawbacks associated with whole blood, mainly donor-dependent availability and potential transmission of viral diseases, while the second can transmit blood-borne diseases

LEH = liposome-encapsulated hemoglobin

LPS = lipopolysaccharide

and may elicit immune reactions. To address this issue, recombinant human hemoglobin was recently produced in bacterial cells [10] and in transgenic pigs [35]. Genetically engineered hemoglobin is a very attractive hemoglobin source that can be incorporated into any delivery system, although problems with large-scale production may hamper the development of such products. As hemoglobin-based red cell substitutes are rapidly evolving for use in clinical practice, it is absolutely necessary to identify the most feasible and reliable source of hemoglobin that will be required for the mass production of these products.

Third, the immunogenicity and immunological consequences of repeated administrations of oxygen-carrying blood products have not yet been addressed in depth. Also, the effect of hemoglobin-based red cell substitutes on the microcirculation is still obscure [36]. In this respect, it is important to elucidate the mechanisms of acellular-hemoglobin-induced vasoconstriction. Furthermore, although preliminary studies have been reported [37,38], it is still unknown whether hemoglobin-derived oxygen-carrying solutions interfere with coagulation systems, function of other blood cells, and typing and cross-matching of donor blood. In addition, safety concerns such as the effect of acellular hemoglobin on renal performance and the effect of LEH on reticuloendothelial system function should be resolved.

Fourth, another problematic issue in the development of hemoglobin-based red cell substitutes is the selection of criteria to test their efficacy. The complexity of trauma patients, and the fact that the time-honored faith in blood transfusion as the mainstay of trauma resuscitation has never been put to a systematic test, clearly complicate this issue. Also, the evolving debate on "dry resuscitation" [39] further questions the appropriate utility and testing of oxygen-carrying resuscitative fluids. Therefore, it is conceivable that conventional placebo-controlled trials, which monitor clinical benefits such as reduced mortality and morbidity as well as improved course of disease, will be very difficult to conduct. Indeed, a recent workshop, jointly sponsored by the National Institutes of Health and the Food and Drug Administration, on criteria for efficacy of red cell substitutes acknowledged this notion and emphasized the use of surrogate endpoints [40]. These endpoints include decreased need for allogenic blood and demonstration of "activity", i.e., demonstration that the product carries oxygen by showing increased arterial oxygen content and hematocrit above those provided by the endogenous hemoglobin [40].

Fifth, a precise definition for the clinical use of oxygen-carrying red cell substitutes has not yet been established, primarily because the safety of these products in patients is still unknown. That is, tolerance to high doses of hemoglobin-based resuscitation fluids will allow these preparations to totally substitute for the oxygen-carrying capacity of conventional donor blood transfusion. In support of such a clinical application, only one product – Polyheme™ - has been shown to be safely infused into patients at doses equivalent to 6 units of packed red blood cells [31]. On the other hand, if large doses of hemoglobin-based oxygen carriers will ultimately be associated with significant toxic side effects, it is conceivable that these red cell substitutes will be applied only to reduce the requirement for allogenic blood when massive transfusion is required. Also, artificial oxygen carriers could then be used as small volume substitutes in a variety of medical indications, including coronary angioplasty, stroke, peripheral vascular disease, anti-tumor therapy, cardiopulmonary bypass and organ preservation.

Summary

There is an urgent need for the development of artificial oxygen-carrying resuscitation fluids. The leading approach in the search for such a product is toward modified or encapsulated hemoglobin, whose development has extended over the last 40 years. Despite significant difficulties – mostly related to safety issues – much progress has been made with several modified free hemoglobin preparations; these are currently undergoing clinical evaluation and human trials, with LEH shortly anticipated. Nevertheless, there are still many unresolved issues on the road to an oxygen-carrying red cell substitute that can be used routinely in place of red blood cells. These include remaining toxicities, immunological effects, definition of ideal properties, establishment of clear guidelines to test clinical safety and efficacy, and identification of clinical applications. To overcome these issues, further commitment from the academia, industry and government is required.

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A diplomat is a man who thinks twice before saying nothing.

Frederick Sawyer