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Clinical Development of Human Polymerized Hemoglobin as a Blood Substitute

Steven A. Gould, M.D.,[1,2,3] Gerald S. Moss, M.D.[2]

[1]Department of Surgery, Columbia Michael Reese Hospital and Medical Center, 2929 S. Ellis Avenue, Chicago, Illinois 60616, U.S.A.
[2]Department of Surgery, University of Illinois, College of Medicine, 1853 W. Polk, Chicago, Illinois 60612, U.S.A.
[3]Northfield Laboratories, Inc., 1560 Sherman Avenue, Suite 1000, Evanston, Illinois 60201, U.S.A.

Abstract. Although the efficacy of hemoglobin-based oxygen carriers was established more than 60 years ago, all prior clinical trials have demonstrated significant toxicity characterized by renal dysfunction, gastrointestinal distress, and systemic vasoconstriction. The mechanisms of these toxicities now appear to be understood. Tetrameric forms of the hemoglobin molecule extravasate from the circulation and interact with endothelium-derived relaxing factor, leading to unopposed vasoconstriction. Although numerous efforts are under way to chemically modify the native tetramer, it is likely that all tetrameric forms of the hemoglobin molecule will continue to extravasate. We have focused on developing a polymerized form of hemoglobin that is virtually free of unreacted tetramer. The development and characterization of this polymerized pyridoxylated hemoglobin solution (Poly SFH-P) is described. Clinical trials have been completed successfully in volunteers and are now under way to assess the safety and efficacy of Poly SFH-P as a clinically useful red blood cell substitute for treatment of acute blood loss in the setting of trauma and surgery.

There continues to be great interest in developing a clinically useful O_2 carrier to serve as a red blood cell (RBC) substitute. The goal is to develop a safe and effective alternative to human blood. Although the current blood supply is safer than ever owing to improved donor screening and testing, it is likely that disease transmission will always occur [1]. This is due to the inevitable occurrence of new viruses and to the small but real incidence of false-negative screening tests, often due to the window period of infectivity prior to conversion of the markers used for screening. In addition, the use of allogeneic blood involves the need for compatibility testing and includes a limited shelf life.

The potential benefits of an RBC substitute include universal compatibility and the accompanying immediate availability, freedom from disease transmission, and long-term storage. The two potential candidates to serve as clinically useful RBC substitutes include hemoglobin solutions and perfluorochemical emulsions [2]. We have had considerable experience with the perfluorochemicals, including a clinical trial in patients with a 20% perfluorochemical emulsion [3–5]. The results of that trial showed that that particular preparation was not effective as an O_2 carrier. The current status of the newer perfluorochemical emulsions is not discussed here. The remainder of this paper deals with the history and development of hemoglobin solutions.

Correspondence to: S.A. Gould, M.D., Northfield Laboratories, Inc., 1560 Sherman Avenue, Suite 1000, Evanston, Illinois 60201, U.S.A.

Background

The basic concept of developing a hemoglobin solution can be illustrated by examining the RBC (Fig. 1). The "active ingredient" in the RBC is the four-part hemoglobin molecule, the tetramer, which chemically binds and carries the oxygen. The hemoglobin molecule has several important characteristics. For example, 1 g of hemoglobin binds 1.39 ml of oxygen and is almost fully saturated with oxygen at ambient pressure. Few if any biologically acceptable substances have a greater oxygen-binding capacity. Oxygen is normally unloaded from hemoglobin in the capillaries at a PO_2 of approximately 40 mmHg, allowing oxygen molecules to diffuse from hemoglobin to the intracellular mitochondria without producing interstitial hypoxia. The physiologic capability of the hemoglobin molecule is clear.

The surrounding cell membrane accounts for the need for compatibility testing and the limited shelf life. It has long been known that although the cell has a finite life-span the hemoglobin protein itself is rather durable and survives and functions outside of the cell. The history of the development of hemoglobin solutions is the tale of efforts to harvest the hemoglobin protein and prepare it in a form that would be safe and useful in the clinical setting [7].

Considerations for a Hemoglobin Solution

Several properties must be considered when describing the nature of a hemoglobin solution. The first is the source of the hemoglobin to be used as the starting material (Table 1). We have always thought that the human source is the best known and most readily available, making it the preferred choice. The bovine source has been proposed as a way of providing easier access to an unlimited supply. The recombinant and transgenic approaches have received considerable interest because of their use of modern molecular biology approaches. However, there is no evidence to suggest any physiologic or functional benefit of using any source other than human hemoglobin. In fact, the choice of source has not been a crucial issue in the development of an RBC substitute.

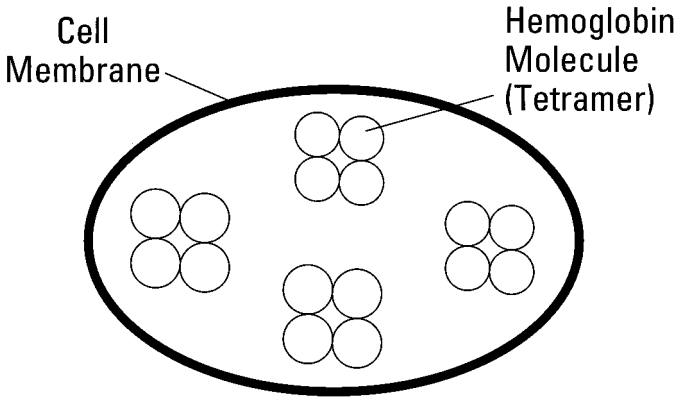

Fig. 1. Red blood cell containing the hemoglobin tetramer. (From Gould et al. [6], with permission.)

Table 1. Hemoglobin sources.

Human
Bovine
Recombinant
Transgenic

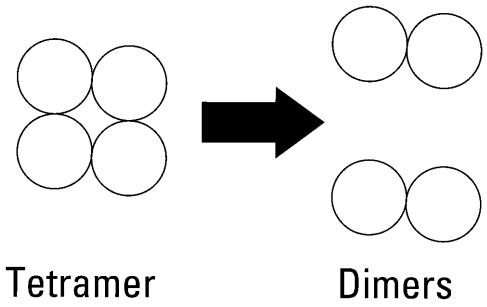

Fig. 2. Unmodified tetramer as it dissociates into dimers. (From Gould et al. [6], with permission.)

The limiting factor in this field has been the safety of the hemoglobin solution. It is now evident that unmodified tetrameric hemoglobin is unsafe because of the associated toxicities of renal dysfunction, gastrointestinal distress, and vasoconstriction [7]. Therefore the most important property of a hemoglobin solution is the nature of the hemoglobin preparation itself. Unmodified tetramer dissociates into dimers when removed from the RBC (Fig. 2). It is now believed that the observed toxicities are due in large part to this dissociation and the presence of free tetramer in the circulation. Efforts to eliminate these toxicities have consisted of a variety of attempts to stabilize the simple unmodified tetramer. Table 2 lists the various preparations. Each effort involves the use of a different approach to try to stabilize the tetramer and thereby avoid toxicity. A conjugated tetramer (Fig. 3) involves the binding of a macromolecule, such as polyethylene glycol (PEG), to form a larger molecule. A cross-linked tetramer (Fig. 4) is an intramolecular chemical or genetic link to stabilize the native hemoglobin molecule. Polymerization involves intermolecular cross-linking of tetramers as shown in Figure 5. The polymerization results in a variety of molecular weight sizes. Finally, encapsulation (Fig. 6) involves "packaging" the hemoglobin molecule within a lipid membrane or liposome to form a "synthetic" RBC. Only testing in humans can eventually resolve the issue of safety with these various preparations. However, it is clear that the nature of the hemoglobin preparation itself is far more important than the choice of source material.

Table 2. Hemoglobin preparation.

Unmodified tetramer
Conjugated tetramer
Cross-linked tetramer
Polymer
Encapsulated molecule

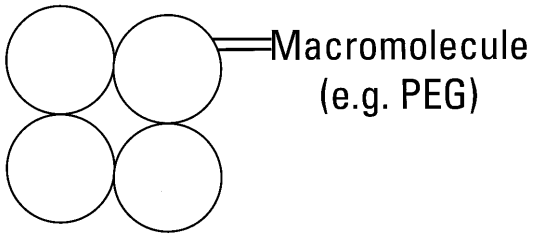

Fig. 3. Conjugated tetramer. PEG: polyethylene glycol. (From Gould et al. [6], with permission.)

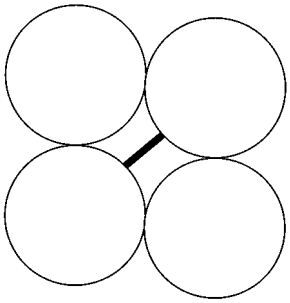

Fig. 4. Cross-linked tetramer. (From Gould et al. [6], with permission.)

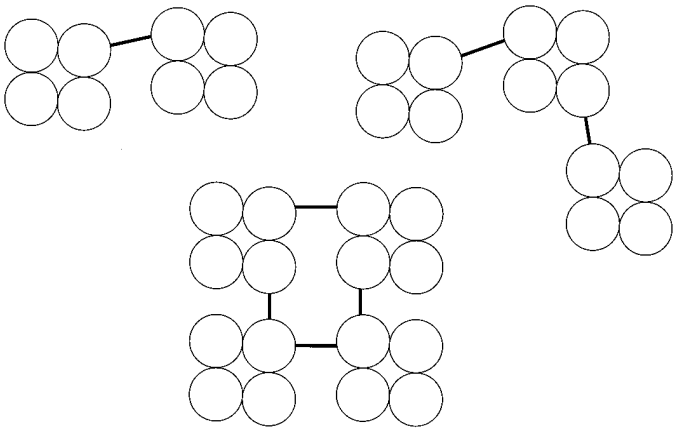

Fig. 5. Polymers composed of two, three, and four tetramers linked together. (From Gould et al. [6], with permission.)

The third characteristic of any hemoglobin solution is the intended clinical use. Table 3 lists some of the potential applica-

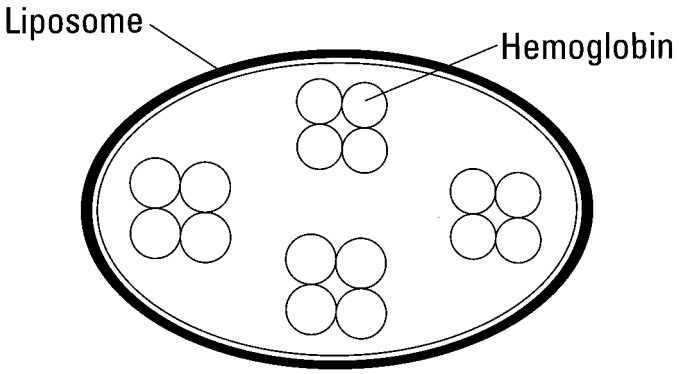

Fig. 6. Liposome-encapsulated hemoglobin. (From Gould et al. [6], with permission.)

Table 3. Hemoglobin clinical use.

Acute blood loss
Hemodilution
Ischemia
Cardioplegia
Organ preservation
Cancer therapy

Table 4. Current approaches.

Human polymer, tetramer-free
Bovine polymer, tetramer present
Human cross-linked tetramer
Recombinant cross-linked tetramer

tions for a safe, effective hemoglobin solution. Although they represent a diverse set of circumstances, it is likely that the major role of a hemoglobin solution is as an RBC substitute for use after acute blood loss.

There are numerous clinical trials under way in volunteers and patients using a variety of these approaches. Ultimately, each effort involves a selection of hemoglobin source, preparation, and intended clinical use. Table 4 shows a list of the source and preparation for each of the current approaches in clinical trials.

Demonstration of Efficacy

The concept of using a hemoglobin solution as an oxygen carrier was first fully tested by Amberson et al. in 1934 [8], who prepared a crude RBC lysate, which was an unmodified tetrameric form of the hemoglobin solution. In a rather remarkable experiment, they gradually removed all of the blood from cats and replaced it with this primitive hemoglobin solution. The results demonstrated the proof of concept and paved the way for all future efforts. The cats survived on a short-term basis and were able to walk normally. In an absolutely elegant maneuver, Amberson et al. evaluated neurologic function by holding the cats on their backs and dropping them. The cats were able to land upright, demonstrating a fully intact nervous system, which is required to perform this complicated neurologic event. The potential utility of hemoglobin solutions was firmly established.

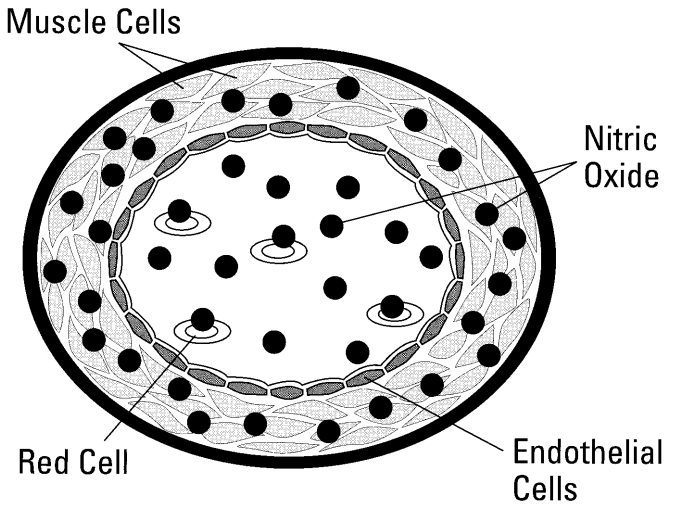

Fig. 7. Control of vasoconstriction. (From Gould et al. [6], with permission.)

Toxicity

Based on the early demonstration by Amberson et al., the efficacy of a hemoglobin solution was uncontestable. However, 60 years later there is still no hemoglobin solution in clinical use. The reason for this lack relates to the results in clinical trials [9–12]. All prior clinical trials through 1978 have been done with unmodified tetrameric forms of hemoglobin solution. These trials have consistently demonstrated major toxicities, consisting of renal dysfunction, gastrointestinal distress, and systemic vasoconstriction characterized by a rise in blood pressure and a fall in heart rate [13]. The Savitsky study in 1978 clearly established the futility of further efforts to develop a safe tetrameric form of hemoglobin solution.

The mechanisms of toxicity of the tetramer are now better understood. In general, these toxicities are related to the presence of the tetramer outside the RBC and the dissociation into dimers. Renal dysfunction is thought to be related to filtration and excretion of the hemoglobin molecule, primarily in the form of dimers, although vasoconstriction is also likely to play a role. The vasoconstriction and other adverse effects are currently thought to be related to extravasation of the tetrameric hemoglobin molecule [14].

Our understanding of the control of vasoconstriction of blood vessels has been improved with the identification of endothelium-derived relaxing factor, also known as nitric oxide [15, 16]. It is well known that nitric oxide avidly binds to hemoglobin. Figure 7 represents the control of vasoconstriction. It is a cross section of a blood vessel representing the lumen, endothelial cell layer, and interstitial space within which reside the vascular smooth muscle cells. Nitric oxide is produced by the endothelial cells and is released on both the luminal and the abluminal sides. The physiologic activity of nitric oxide occurs on the vascular smooth muscle cells in the interstitial space. The nitric oxide released into the lumen is rapidly bound by the hemoglobin within the circulating RBCs and does not act on the smooth muscle cells. It is also evident that RBCs do not normally move beyond the endothelial cell lining into the interstitial space, leaving the nitric oxide available to exert its vasorelaxant effect.

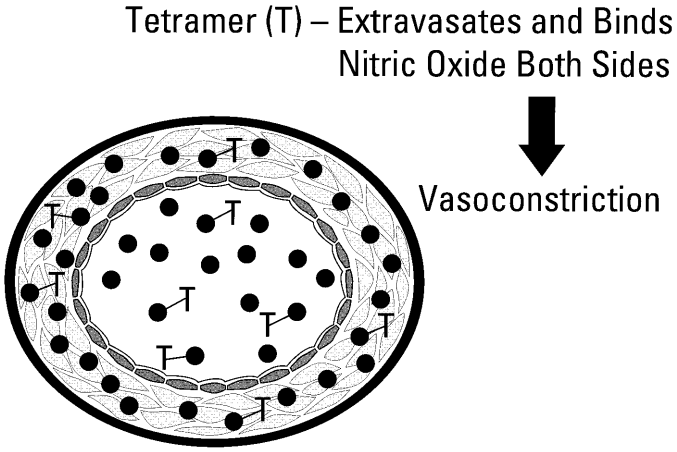

Fig. 8. Effect of a tetramer on extravasation and binding of nitric oxide. (From Gould et al. [6], with permission.)

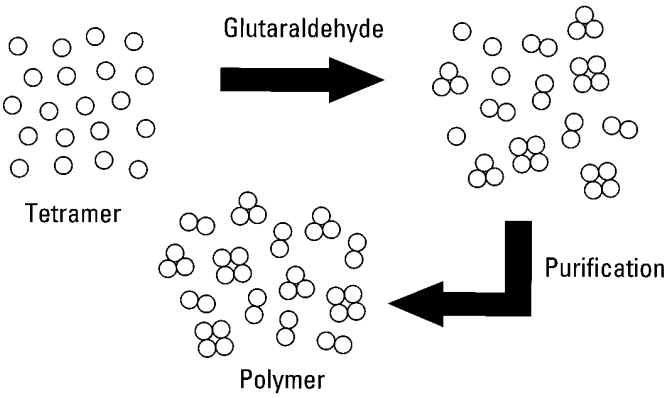

Fig. 10. Preparation of Poly SFH-P, including polymerization and removal of unreacted tetramer (purification). (From Gould et al. [6], with permission.)

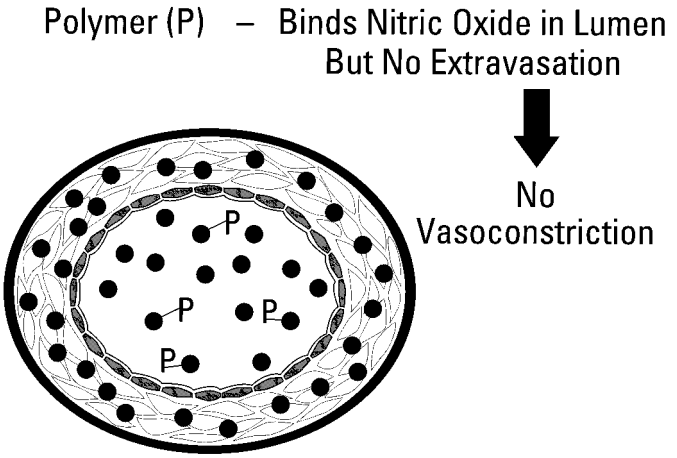

Fig. 9. Polymer and absence of extravasation. (From Gould et al. [6], with permission.)

The vasoconstriction that occurs with the tetrameric form of the hemoglobin is considered to be due to extravasation of the hemoglobin beyond the endothelial cell barrier and the immediate binding of the nitric oxide to hemoglobin, leading to unopposed vasoconstriction. This sequence is illustrated in Figure 8. The presence of the tetramer in the interstitial space where nitric oxide normally acts thereby disrupts the normal control of vascular relaxation. Our working hypothesis has been that prevention of extravasation prevents vasoconstriction, as nitric oxide would function normally. It has also been our contention that all forms of tetramer extravasate and produce both vasoconstriction and the other toxicities historically associated with hemoglobin solutions [14, 17, 18]. The only forms of hemoglobin that appear to prevent extravasation include the polymer and encapsulation. The cross-linked tetramers have indeed been shown to extravasate [14, 18]. Because the encapsulation approach is still in the developmental stage, only the polymer has been able to be evaluated in the clinical setting. Figure 9 illustrates the likely scenario when the polymer is infused. As with the RBC, the polymer exists only in the lumen, leaving the nitric oxide to exert its normal physiologic vasorelaxant role on the vascular smooth muscle cell in the interstitial space.

Our approach therefore has been specifically to design a hemoglobin solution using a two-step process to create a tetramer-free form of polymerized hemoglobin [19–22]. The first step is polymerization, which results in an array of different size polymers. The second step, which is just as important, is the removal of all unreacted tetramer. Figure 10 is a representation of this process. Glutaraldehyde is used as the cross-linking (polymerizing) agent. The second step is to remove virtually all unreacted tetramer, leading to a pure polymeric solution. The remainder of this paper deals with the historical development of our human polymerized hemoglobin solution, including the physiologic observations that led to the characteristics of our current preparation.

Unmodified Hemoglobin

The basic, unmodified hemoglobin solution is currently prepared from outdated blood, beginning with the washing and lysis of the RBCs with pyrogen-free water. A series of filtration steps permits complete separation of the RBC membrane debris (stroma) from the hemoglobin molecules. The resultant solution is referred to as stroma-free hemoglobin (SFH). Because the RBC antigens are located on the cell membrane, SFH is universally compatible and can be infused without regard to specific blood type. The properties of this unmodified, tetrameric, or "stripped" hemoglobin solution are given in Table 5.

Although SFH can be prepared with a hemoglobin concentration of 14 g/dl, this solution has a colloid osmotic pressure (COP) of more than 60 mmHg, which renders it unacceptable for clinical use [23]. The hemoglobin concentration of 7 g/dl is isooncotic. The low P_{50} is due to the loss of the organic ligand 2,3-diphosphoglycerate (2,3-DPG) during preparation. The $[O_2]$ curve of SFH is thus both anemic (decreased hemoglobin content) and leftward-shifted (decreased P_{50}).

Despite these limitations, SFH supports life in primates in the absence of RBCs [23]. Animals survive a total exchange transfusion with SFH to zero hematocrit with maintenance of normal O_2 consumption ($\dot{V}O_2$), cardiac output, and arteriovenous oxygen

Table 5. Properties and parameters of stroma-free hemoglobin and whole blood.

Properties and parameters	Stroma-free hemoglobin	Whole blood
Hemoglobin content (g/dl)	6–8	12–14
Oxygen-carrying capacity (vol%)	8–11	16–19
Binding coefficient (ml O_2/g Hb)	1.30	1.30
P_{50} (mmHg) (PCO 40 mmHg, pH 7.40)	12–14	26–28
Methemoglobin (%)	< 2	< 1
Colloid osmotic pressure (mmHg)	18–25	18–25
Osmolarity (mOsm)	290–310	290–310

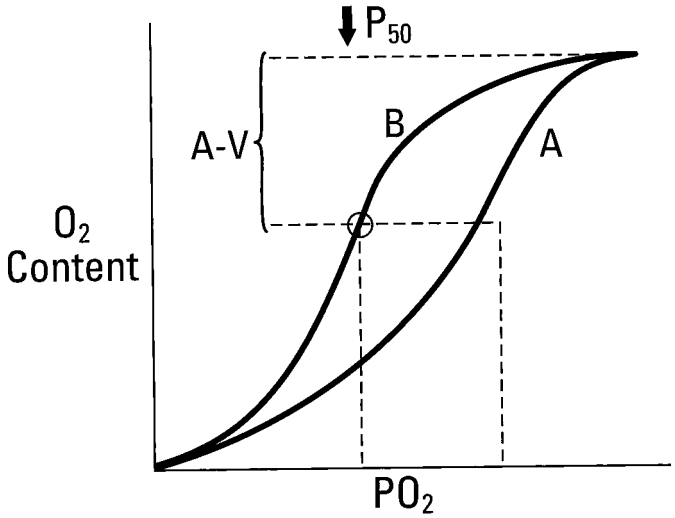

Fig. 11. $[O_2]$ curves showing a leftward shift in P_{50} with a constant $CaO_2 - CvO_2$ that leads to a lower $P\bar{v}O_2$. Curve B is shifted to the left compared to curve A. (From Moss et al. [25], with permission.)

content difference ($CaO_2 - CvO_2$), although a decline from baseline values occurs in some of these measures. In addition, a considerable decrease occurs in the $P\bar{v}O_2$ from roughly 50 mmHg to 20 mmHg. The $P\bar{v}O_2$ is the partial pressure at which oxygen unloads from the hemoglobin molecule; it is in equilibrium with the tissue PO_2. This decline indicates a marked increase in oxygen extraction and is the mechanism used to compensate for the fall in hemoglobin content and P_{50}. This low $P\bar{v}O_2$ is of some concern and led us to attempt to restore a more normal value [24].

Pyridoxylated Hemoglobin

A leftward shift in the $[O_2]$ curve, with no change in $\dot{V}O_2$, cardiac output, or $CaO_2 - CvO_2$, produces a decrease in the $P\bar{v}O_2$ (Fig. 11). Attempts to establish a normal P_{50} by the simple addition of 2,3-DPG to the hemoglobin solution were unsuccessful because the ligand disappears rapidly from the circulation after infusion. However, modification of the hemoglobin molecule by adding pyridoxal phosphate results in a pyridoxylated hemoglobin (SFH-P) with a P_{50} of 20 to 22 mmHg, which is considerably higher than the P_{50} of unmodified SFH [26–28].

We evaluated SFH-P in eight baboons [29]. Four received SFH and four received SFH-P, with a final hemoglobin content of 7 g/dl and zero hematocrit. The $P\bar{v}O_2$ levels were significantly higher at the end of the exchange in the animals receiving SFH-P. Although hemodynamic parameters were normal, a decline from the baseline values occurred in both groups.

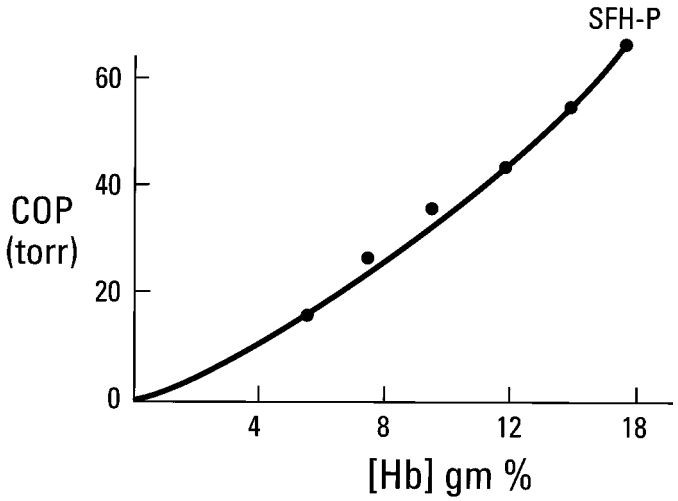

Fig. 12. Relation between colloid osmotic pressure (COP) and hemoglobin concentration for SFH-P. (From Moss et al. [25], with permission.)

These results illustrate three points. First, a rightward shift in the dissociation curve (increased P_{50}) results in increased $P\bar{v}O_2$ because all else remained constant. This observation is of physiologic importance because oxygen unloading can occur at a higher tissue PO_2. Second, although it was increased, the $P\bar{v}O_2$ level in the animals treated with SFH-P was still substantially lower than the normal value of 40 to 50 mmHg found in control animals. Third, hemodynamic function still showed a reduction from the baseline values. It became apparent that a nonanemic hemoglobin solution was required [24].

Nonanemic Isooncotic Hemoglobin Solution

The advantages of a nonanemic hemoglobin solution are self-evident. Such a solution would have the same oxygen capacity as whole blood. In addition, according to our data the infusion of a nonanemic solution should be associated with normal $P\bar{v}O_2$ levels, even at zero hematocrit [24]. The principal obstacle to normalization of hemoglobin concentration is the effect of an elevation in protein concentration on oncotic pressure.

The relation between hemoglobin concentration and oncotic pressure is shown in Figure 12. At hemoglobin concentrations of 7 g/dl, the oncotic pressure is similar to that of plasma (20 mmHg). In contrast, at hemoglobin levels of 15 g/dl the oncotic pressure increases by more than 300%. The infusion of such a solution would theoretically produce large shifts of fluid from the extravascular space into the intravascular space. These changes are likely to be harmful.

One approach to producing a nonanemic hemoglobin solution with normal COP values is polymerization of the hemoglobin. The COP of any solution is proportional to the number of colloidal particles. If a 15 g/dl solution of hemoglobin could be polymerized, the result would be a reduction in COP but no change in

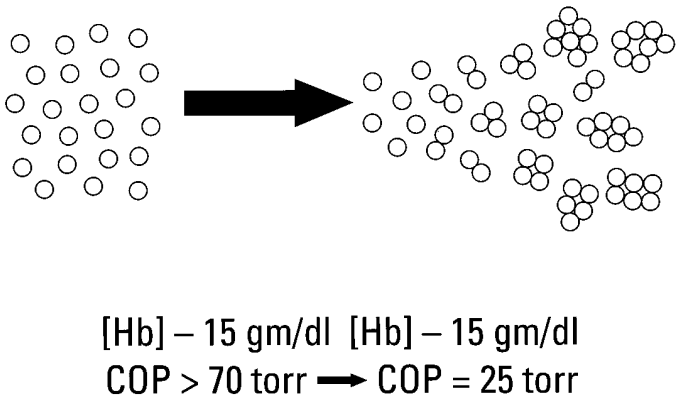

Fig. 13. Polymerization. (From Moss et al. [25], with permission.)

hemoglobin concentration (Fig. 13). This idea was tested in our laboratories [19]. The hemoglobin solution obtained was pyridoxylated by modification of previously described techniques. The goal of the polymerization is to normalize the oxygen capacity while maintaining the COP within normal limits (20–25 mmHg). The characteristics of the final product are listed in Table 6.

Table 6. Properties of polymerized pyridoxylated hemoglobin.

Property	Value
Hemoglobin (g/dl)	12–14
Oxygen-carrying capacity (vol%)	16–19
Methemoglobin (%)	< 5
Molecular weight (daltons)	
Range	64,000–400,000
Average	150,000
P_{50} (mmHg)	18–22
Binding coefficient (ml O_2/g Hb)	1.30
Colloid osmotic pressure (mmHg)	20–25

Efficacy of Poly SFH-P

Seven adult baboons were anesthetized, paralyzed, intubated, and mechanically ventilated with room air [30, 31]. The respiratory rate and tidal volume were adjusted to maintain a $PaCO_2$ between 35 and 45 mmHg before the start of the study and were not changed during the study. The animals were surgically prepared with arterial and central venous catheters for infusion, blood sampling, and monitoring. A thermal dilution balloon-tipped catheter was floated into the pulmonary artery. A Foley catheter was inserted into the urinary bladder. Standard hemodynamic monitoring was performed by electrocardiogram, arterial pressures, pulmonary capillary wedge pressure, and central venous pressure. Cardiac output was determined by the thermal dilution method.

The study was conducted with the use of ketamine anesthesia. After stabilizing the animals, a set of baseline measurements was obtained. An isovolemic exchange transfusion with the Poly SFH-P was then performed. Whole blood was removed in 50-ml aliquots and was replaced with approximately equal volumes of the infusate. Additional volume adjustments were made as required to maintain the pulmonary capillary wedge pressure at baseline values. The exchange was stopped at hematocrits of 20%, 10%, and 5% to obtain additional sets of measurements. The exchange transfusion was then carried out to obtain a complete washout of the RBCs. A hematocrit of less than 1% was achieved.

These animals, at zero hematocrit, had a Poly SFH-P concentration of approximately 10 g/dl. They were then exchanged-transfused with dextran 70 to a hemoglobin concentration of 1 g/dl [32]. The data from the second half of the study were compared with those from a control group ($n = 6$) that underwent an exchange transfusion with dextran 70 to a hemoglobin concentration of 1 g/dl.

The efficacy of the Poly SFH-P was calculated as we have previously described [33]. At each hematocrit level, the arterial $[O_2]$($[O_2]_a$) was determined for each compartment by direct measurement or calculation. Total VO_2 was calculated as the product of the cardiac output and $CaO_2 - CvO_2$. The contribution of Poly SFH-P to oxygen delivery was calculated as the Poly SFH-P/total arterial $[O_2]$ ratio.

$$\text{Poly SFH-P } O_2 \text{ delivery} = \frac{[O_2], \text{Poly SFH-P}}{[O_2]_a, \text{total}}$$

The contribution of Poly SFH-P to VO_2 was calculated as the Poly SFH-P/total $CaO_2 - CvO_2$ ratio.

$$\text{Poly SFH-P } VO_2 = \frac{\text{Poly SFH-P } (CaO_2 - CvO_2)}{\text{total } (CaO_2 - CvO_2)}$$

All contributions were expressed as their percent values.

All animals receiving Poly SFH-P survived the exchange transfusion, as did the previous animals receiving SFH-P. The final hematocrit was 0.8 ± 0.4% (mean ± SEM). The difference in the initial bag P_{50} values of the two infusates was statistically significant ($p < 0.05$). However, the mean in vivo plasma P_{50} for the Poly SFH-P was 17.0 ± 0.5 mmHg, which was not significantly different from the mean value of 17.6 ± 0.8 mmHg for the SFH-P. Both of these plasma P_{50} values are significantly below the mean baboon RBC P_{50} of 31.3 ± 0.8 mmHg. The Poly SFH-P $[O_2]_a$ is significantly greater than the SFH-P value at all hematocrits ($p < 0.001$). At a hematocrit of 5%, the $[O_2]_a$ was 9.5 ± 0.2 vol% for Poly SFH-P and 5.0 ± 0.4 vol% for SFH-P. The percent contributions of Poly SFH-P to total oxygen delivery and total VO_2 were compared with those of SFH-P. Poly SFH-P makes a significantly greater ($p < 0.2$) contribution to total oxygen delivery than SFH-P at all hematocrits. The contribution to total VO_2 is greater by Poly SFH-P at all hematocrits, with the difference significant ($p < 0.005$) at a hematocrit of 20%. These results document that Poly SFH-P is an effective O_2 carrier and provides more benefit than the tetrameric form of SFH-P.

The in vivo P_{50} in the Poly SFH-P animals undergoing the second exchange transfusion with dextran 70 ranged from 18 to 11 mmHg. In contrast, the in vivo P_{50} of the control group ranged from 31.5 to 25.5 mmHg. The $P\bar{v}O_2$ was significantly lower in the Poly SFH-P group compared with the control group. Both groups of animals raised their cardiac output in an identical manner in response to their anemia. The critical oxygen delivery in the control group was 6.6 ml/min/kg body weight compared with 5.7 ml/min/kg in the test group. These results indicate that the infusion of Poly SFH-P does not alter the normal physiologic response to progressive anemia and therefore provides evidence of lack of vasoconstriction.

Table 7. Characteristics of Poly SFH-P for clinical trials.

Property	Value
Hb	10 g/dl
P_{50}	28–30 mmHg
Met Hb	< 3%
Tetramer	< 1%
1 Unit = 500 ml (50 g)	

Table 8. Clinical experience with 30 healthy volunteers.

Parameter	Preinfusion	Postinfusion
Heart rate	63 ± 8	60.0 ± 9.0
Mean arterial pressure	95 ± 12	100 ± 13
Inulin clearance (ml/min/m^2)	52 ± 13	51 ± 12

Clinical Trials

Based on these preclinical observations we have begun clinical trials in both healthy volunteers and patients to assess the safety and efficacy of Poly SFH-P. For these trials a decision was made to prepare the Poly SFH-P in a fashion that would allow 1 unit of Poly SFH-P to deliver the equivalent amount of hemoglobin contained in a 1-unit blood transfusion. Therefore each unit contains 500 ml at a 10 g/dl concentration, thereby delivering 50 g of hemoglobin. In addition, continued improvement in the process enabled the P_{50} to be increased to 28 to 30 mmHg. The characteristics of 1 unit of Poly SFH-P used for clinical trials is shown in Table 7. To date the clinical trials have included healthy volunteers [34], stable patients, and patients being resuscitated from hemorrhagic shock following major trauma [35].

The Poly SFH-P was successfully infused in doses up to the equivalent of a 1-unit transfusion of blood (50 g) in healthy volunteers without the undesirable effects historically associated with hemoglobin solutions, including vasoconstriction, kidney dysfunction, or gastrointestinal distress (Table 8). This experience provided sufficient evidence to begin therapeutic trials with Poly SFH-P for treating the acute blood loss that occurs following trauma or during surgery.

The patient protocol involves infusing up to 3 units (150 g) in patients suffering acute blood loss after major trauma and during major surgery. The clinical investigators evaluate the patients in the traditional manner. When a clinical decision is made that transfusion is indicated, the patient then receives 1, 2, or 3 units of Poly SFH-P as necessary in lieu of RBCs. Thirty patients (21 men, 9 women) 19 to 83 years of age have received 1 unit (n = 14), 2 units (n = 2), or 3 units (n = 14) of Poly SFH-P (150 g) instead of RBCs as part of their blood replacement. As with the volunteers there have been no safety concerns. We have begun to address efficacy [36]. In the presence of RBCs and Poly SFH-P, the total concentrations of hemoglobin is expressed as:

$$\text{Total [Hb]} = \text{RBC [Hb]} + \text{Poly SFH-P [Hb]}$$

The data for plasma [Hb] (representing the Poly SFH-P), total [Hb], and RBC [Hb] are shown in Table 9. Each infusion of a unit of 50 g of Poly SFH-P increases the plasma [Hb] about 1 g/dl, similar to the 1 g/dl increase in [Hb] that typically occurs after infusion of 1 unit of allogeneic blood. Furthermore, 12 of the 30 patients who would otherwise have required blood received no allogeneic transfusion during the first 24 hours following their injury.

Table 9. Clinical experience with 30 patients.

Time of measurement	Plasma Hb (g/dl)	Red blood cell Hb (g/dl)	Total Hb (g/dl)
Preinfusion	0	9.7 ± 2.3	9.7 ± 2.3
After 1 unit	1.3 ± 0.4	8.6 ± 1.8	9.5 ± 1.6
After 2 units	2.6 ± 0.7	6.4 ± 1.2	8.6 ± 0.9
After 3 units	3.5 ± 0.8	5.6 ± 1.1	8.6 ± 0.4

Although the work is still in progress at doses up to 6 units of Poly SFH-P, these results appear to confirm the safety and efficacy observations from the preclinical studies. The protocol and design seem appropriate. Poly SFH-P is being used exactly like blood in the most relevant setting of urgent hemorrhage. This therapy with Poly SFH-P provides a benefit to the patient by reducing allogeneic transfusion during the treatment of hemorrhage. Poly SFH-P therefore appears to be a clinically useful blood substitute. Trials continue at increased doses and in a randomized, controlled study to compare safety and efficacy to that of allogeneic blood. The outcome of these studies will ultimately help determine the proper role for a blood substitute in the care of the injured patient.

Résumé

Bien que l'efficacité des transporteurs d'oxygène basés sur l'hémoglobine soit établie depuis plus de 60 ans, tous les essais cliniques antérieurs ont démontré une toxicité importante associant l'insuffisance rénale, des désordres gastro-intestinaux et une vasoconstriction systémique. Les mécanismes de cette toxicité paraissent aujourd'hui connus. Les formes tétramériques de l'hémoglobine sont caractérisées par leur extravasation et interagissent avec un facteur relaxant dérivé de l'endothélium, créant une vasoconstriction irréversible. Bien que de nombreux efforts sont actuellement en cours pour modifier chimiquement le tétramère natif, il semble que, quelle que soit la forme tétramérique des molécules d'hémoglobine, elles vont continuer de s'extravaser. Nous avons concentré nos efforts pour mettre au point une forme polymérisée d'hémoglobine qui est virtuellement libre de tétramère actif. Le développement et les caractéristiques de cette solution d'hémoglobine pyridoxylée polymérisée (poly SFH-P) sont décrits. Des essais cliniques chez des volontaires sont des succès et nous sommes en train d'évaluer la sûreté et l'efficacité de la poly-SFH comme substitut clinique de globules rouges dans le traitement de l'anémie aiguë en traumatologie et en chirurgie.

Resumen

Aunque la eficacia de transportadores de oxígeno basados en hemoglobina fue establecida hace más de sesenta años, todos los ensayos clínicos previos han demostrado una toxicidad significativa que se caracteriza por disfunción renal, dificultad gastrointestinal y vasoconstricción sistémica. Los mecanismos de tales efectos tóxicos ahora aparecen comprensibles. Formas tetraméri-

cas de la molécula de hemoglobina se extravasan de la circulación e interactúan con el factor relajante derivado del endotelio, dando lugar a vasoconstricción no controlada. A pesar de los numerosos esfuerzos que se realizan actualmente para modificar químicamente el tetrámero nativo, es probable que todas las formas tetraméricas de la molécula de hemoglobina continúen extravasando. Hemos enfocado nuestro esfuerzo hacia el desarrollo de una forma polimerizada de la hemoglobina que está virtualmente libre del tetrámero no reactivo. Se describen el desarrollo y la caracterización de esta soluci ón polimerizada de hemoglobina piridoxilada (Poly SFH-P). Se han completado ensayos clínicos exitosos en voluntarios, y se efectúan ensayos para determinar la seguridad y eficacia de la Poly SFH-P como un sustituto clínicamente útil de los glóbulos rojos en el tratamiento de la pérdida aguda de sangre, en el contexto del trauma y la cirugía mayor.

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