Potential Uses of Hemoglobin-based Oxygen Carriers in Critical Care Medicine

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KEYWORDS

• Oncotic pressure • Nitric oxide • Sepsis
• Perioperative blood loss • Transfusion
• Oxygen transport • Red blood cell • Trauma

The two major impulses driving the development of hemoglobin-based oxygen carriers (HBOCs) are concerns about the infectious risks of transfusion and worries about limitations in the availability of blood. Almost 40,000 red blood cell (RBC) transfusions are given each day in the United States, and about 5 million Americans receive transfusions every year for a variety of reasons. The volume of blood transfused is increasing at the rate of 6% per year, and the greatest fear of the blood bank community at present is impending shortages. Although seasonal and regional shortages are not uncommon, an extended, nationwide shortage of blood has not been observed in recent history, even though about 6% of United States hospitals have reported that surgical procedures have had to be cancelled or postponed because of blood shortages.[1] A shortage of RBCs may occur, however, if transfusion demand continues to increase and if collections cannot keep pace.[1]

Considerable progress has been made in developing HBOC products that meet many of the criteria for a clinically useful and safe oxygen carrier, including better shelf stability than banked RBCs, universal compatibility, useful vascular half-life, and absence of infectious agents (**Box 1**). The HBOCs under development all have vascular half-lives in the 18- to 24-hour range, which is adequate for most acute-care applications (ie, hemorrhage and surgery); most can be stored at 4°C or room temperature for 1 to 2 years; and none requires any form of compatibility testing. All the HBOCs currently developed have been successfully processed to eliminate the presence of microorganisms, although there are very few published data on the removal of prions.

With the realization that HBOCs not only are RBC substitutes but also have many other properties, including hemodynamic effects related to their oncotic and nitric

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Box 1
Potential advantages of HBOCs over RBC transfusion

No antigenicity—universally compatible

Possible unlimited availability (animal or recombinant source)

No disease-transmission risk

Long storage life

oxide (NO)-scavenging effects, a new, broader concept of "hemoglobin therapeutics" has developed. HBOCs, therefore, are now seen as a complement to RBC transfusions, not a replacement. The present review focuses on the potential uses of HBOCs in critical care medicine.

PROPERTIES OF HEMOGLOBIN-BASED OXYGEN CARRIERS

Oxygen-Carrying Capacity

Hemoglobin concentration, hemoglobin saturation, and blood flow are key determinants of tissue oxygen availability, so strategies to increase systemic oxygen transport (convective oxygen transport—ie, circulation) are usually employed when attempting to increase tissue oxygen availability. Nevertheless, these factors are not the only determinants of tissue oxygenation in critical illness.[2] Sepsis is characterized by microcirculatory alterations[3] that can lead to tissue hypoxia, despite the presence of elevated convective oxygen transport. Even with a high oxygen-carrying capacity, a solution that alters the microcirculation is not efficient at improving tissue oxygenation. The different types of HBOCs have very different hemodynamic properties (vasoconstrictive and oncotic effects), so their effects on the microcirculation may be very different. In theory, a small amount of a hemoglobin solution with no vasopressor effects is more effective in improving tissue oxygenation than a large amount of a vasopressor solution that alters microcirculatory blood flow. Nevertheless, in general, compared with nonhemoglobin solutions, resuscitation fluids containing hemoglobin improve oxygen transport to the tissues.[4–10]

Oncotic Effects

RBCs have a negligible effect on the colloid osmotic pressure (COP). HBOCs, however, exert substantial oncotic properties and thus increase blood volume by an amount greater than the transfused volume.[11] This action may be beneficial when plasma expansion is required in shock resuscitation but can also be harmful in the absence of hypovolemia. It has been proposed that hyperoncotic HBOCs may decrease ischemic brain injury.[12] Nevertheless, the beneficial effects of a reduction in blood viscosity on microvascular blood flow may also be important. Using bioengineering analysis and novel methods to measure intravascular and tissue P_{O_2}, Intaglietta and colleagues[13] proposed that maintaining the viscosity of blood ($\sim$4 centipoise) is essential to maintain tissue oxygenation. Indeed, the relation between blood viscosity and vascular relaxation is explained by the fact that the shear forces exerted on endothelial cells are proportional to blood viscosity, and these shear forces induce the production of the vasodilator, NO.[14,15] Tsai and colleagues[16] showed that deep hemodilution with high viscosity dextran maintained functional capillary density in the hamster skin-fold model significantly better than with low viscosity dextran. These discoveries force a re-evaluation of the optimal viscosity of an RBC substitute. It was formerly thought

that such a solution should have a low viscosity to reduce peripheral vascular resistance and to elevate cardiac output; now, the opposite appears to be the case.

Vasopressor Effects

Many experimental studies have reported a rise in blood pressure following the administration of HBOCs,[5,7,10,17–22] and clinical trials have confirmed this observation.[9,23–29] These vasopressor effects are, at least in part, due to the scavenging of NO.[19,30–35] The NO-scavenging effects may be mitigated by encapsulating the hemoglobin in liposomes, because these larger molecules have less contact with the endothelium and are less able to penetrate the endothelium to combine with NO.[36] Endothelin-1, a strong vasoconstrictor produced by endothelial cells and acting directly on vascular smooth muscle, may also be involved in the vasoconstrictor effects of hemoglobin solutions.[22,31,37,38] Nevertheless, effects other than NO scavenging must be involved. Indeed, some investigators[39] have demonstrated that various types of modified hemoglobins (cross-linked, polymerized, or surface-modified) elicit various blood pressure responses that are inversely related to the size of the hemoglobin molecule but not to the degree of reactivity with NO. Sakai and colleagues[40] studied the effects of different HBOCs of various molecular sizes on the diameter of resistance arteries and arterial blood pressure in the conscious hamster dorsal skin-fold model. These investigators found that the vasoconstrictive response of resistance arteries was correlated with the degree of hypertension and that the responses were proportional to the molecular dimensions of the oxygen carriers. These observations are best explained by the relation between molecular size, oxyhemoglobin diffusion, and vasoconstriction. It has long been known that oxyhemoglobin can diffuse along a concentration gradient, a process called "facilitated diffusion."[41,42] It has been proposed that this oxygen availability could be significantly increased by free plasma hemoglobin. One group of investigators showed that administration of such an HBOC increased pulmonary diffusing capacity.[9] At first glance, this facilitated oxygen transfer might be considered beneficial; however, direct observation of the microcirculation has shown that precapillary arterioles are very sensitive to local oxygen concentration, enabling them to adjust their diameter in an autoregulatory process to increase capillary blood flow in hypoxia and to decrease it in hyperoxia.[43] Using direct measurement of the diffusive oxygen transfer by hemoglobin in an artificial capillary system, McCarthy and colleagues[44] confirmed the link between molecular size, diffusion, and vasoconstriction. These investigators demonstrated that the rate of oxygen transfer out of the in vitro microvessel was higher with cross-linked hemoglobin than with larger-size surface-modified hemoglobin, such as polyethylene glycol (PEG)-modified bovine hemoglobin (PEG-Hb), which has negligible vasoactive effects.[39,45] Indeed, Winslow and colleagues[45] compared the effects of PEG-Hb (high oxygen affinity, high viscosity, high COP) with human hemoglobin cross-linked between the α-chains ($\alpha\alpha$-Hb; low oxygen affinity, low viscosity, low COP) after a 50% (by volume) exchange transfusion followed by severe hemorrhage in rats. The investigators found that mean arterial pressure and systemic vascular resistance rose significantly in the $\alpha\alpha$-Hb but not in the PEG-Hb animals. In addition, the PEG-Hb animals showed no acid-base disturbance, significantly less lactic acidosis, and a higher cardiac output. These data suggest that the rise in vascular resistance that follows the $\alpha\alpha$-Hb exchange transfusion offsets the greater oxygen transport provided by the cell-free hemoglobin. When resistance does not rise, as with PEG-Hb, even relatively small amounts of cell-free hemoglobin appear to provide very effective blood replacement. Such beneficial effects of a hemoglobin solution on the microcirculation were demonstrated by Wettstein and colleagues,[46] who resuscitated hamsters from severe hemorrhagic shock

with a new PEG-modified human hemoglobin solution—malPEG-Hb, an oxygen-carrying blood replacement fluid with low hemoglobin concentration, high viscosity, high COP, and very low P50 (5 mm Hg). Compared with resuscitation with shed blood or colloid, the infusion of malPEG-Hb was followed by an improvement in microcirculatory blood flow (increased functional capillary density) and microcirculatory oxygen extraction capabilities, resulting in a complete reversal of metabolic acidosis. This result was not seen in animals resuscitated with shed blood or colloid, even though the total plasma hemoglobin concentration was lower in the malPEG-Hb than in the shed blood group. Again, this study[46] seems to underline the efficacy of a solution that associates high viscosity, high COP, and high oxygen affinity properties to maintain microvascular blood flow and tissue oxygenation. In this case, even a low-dose oxygen carrier may be superior to autologous blood in returning the organism to normal conditions after hemorrhagic shock.[46] Properties of first- and second-generation HBOCs are summarized in **Table 1**.

USE OF HEMOGLOBIN-BASED OXYGEN CARRIERS AS A BLOOD SUBSTITUTE

A blood substitute that eliminates the need for refrigeration and cross-matching, that has a long shelf life, and that reduces the risk of iatrogenic infection would provide a potentially lifesaving option for trauma patients who have hemorrhagic shock, especially in rural areas and military settings. For their use in the hospital, blood substitute HBOCs should be at least as safe as RBCs transfusions, even though the risks associated with transfusion have become relatively low in developed countries. At locations where RBCs are not immediately available, the risks of HBOCs need to be balanced against those of resuscitation fluids. One topic of active debate over the past decade was the definition of ''efficacy'' of HBOCs. Demonstrating survival benefit in most clinical models is very challenging, and correlating oxygen delivery and other variables with survival outcome is equally difficult. Therefore, avoidance of allogeneic transfusion has almost become a universal marker of efficacy for these solutions in clinical trials.

Trauma and Hemorrhagic Shock

Because of their hemodynamic properties (see earlier discussion), HBOCs would appear to be ideal solutions for initial resuscitation from trauma or hemorrhage in the emergency room and even in the field. Gould and colleagues[47] compared the therapeutic benefit of PolyHeme (a human polymerized hemoglobin; Northfield Laboratories, Inc., Evanston, Illinois) with that of allogeneic RBCs in the treatment of acute blood loss in trauma patients. PolyHeme maintained total hemoglobin concentration despite the fall in RBC hemoglobin, and reduced the use of allogeneic blood. Later, PolyHeme was studied by the same group in massively bleeding trauma and urgent surgery patients who did not receive RBCs.[48] A total of 171 patients received a rapid

Table 1
Properties of first- and second-generation hemoglobin-based oxygen carriers

Property	First Generation	Second-Generation
Oxygen binding	Like blood: P50 ±28 mm Hg	P50 ±5–10 mm Hg
Viscosity	Like water: ±1 centipoise	Like blood: ±4 centipoise
Oncotic pressure	Like blood: ±15 mm Hg	Increased
Hemoglobin concentration	Like blood: ±15 g/dL	As low as possible
Plasma retention	As long as possible	As long as possible
Clinical use	Substitute for RBCs	Hemoglobin therapeutics

infusion of 1 to 20 units (1000 g, 10 L) of PolyHeme. Forty patients had a nadir RBC hemoglobin below 3 g/dL, but total hemoglobin was adequately maintained (6.8 ± 0.7 g/dL) by the plasma hemoglobin added by PolyHeme. The 30-day mortality was 25% compared with 64% in a historical group of control patients who had similar RBC hemoglobin levels. Less encouraging were the results of the pivotal phase III trauma trial with the Baxter Healthcare Corporation (Baxter) diaspirin cross-linked hemoglobin (DCLHb) product.[49] This United States multicenter trial was designed to determine whether the infusion of up to 1000 mL of DCLHb during initial hospital resuscitation could reduce 28-day mortality in traumatic hemorrhage. The study was stopped prematurely after the enrolment of 112 patients when an intermediate analysis showed an increased 28-day mortality in the DCLHb-resuscitated group compared with the control group (46 versus 17%, respectively; $P = .003$). The investigators could not correlate the excessive mortality with any specific morbidity but reported that the DCLHb treatment group showed a higher incidence of prehospital cardiac arrest and traumatic brain injuries. They concluded that the vasopressor effect could have contributed to the untoward effect of DCLHb treatment, either by accelerating bleeding or by tissue vasoconstriction, both of which would decrease tissue perfusion. Baxter conducted a companion phase III trauma trial in Europe using morbidity, rather than mortality, as the efficacy end point.[50] Another important difference was that in the European study, the patients were already randomized ''on the scene'' to receive DCLHb or standard fluid therapy. Organ failures and survival rates until day 5 and day 28 showed no significant differences. The median volumes of cumulative blood products administered on day 1 (1595 versus 3716 mL) and day 7 (3139 versus 4746 mL) were lower in the DCLHb group than in the control group. The trial, however, was discontinued after 121 of the expected 400 to 800 patients were enrolled because Baxter terminated the program. Nevertheless, it should be noted that the properties of DCLHb (and particularly the strong vasopressor effect) are not applicable to all HBOCs. Northfield Laboratories last year reported results of its pivotal phase III trauma trial with PolyHeme[51] on 30-day mortality in traumatic shock. Severely injured patients were randomized at the scene to receive PolyHeme or crystalloid. The study group received up to 6 units (in the field or during the first 12 hours of hospitalization) of PolyHeme, followed by RBC transfusions as needed. The study was designed to have sufficient statistical power to demonstrate superiority and noninferiority end points. The secondary end points included incidence of multiple organ failure and avoidance of blood transfusion. The study population consisted of 722 patients at more than 30 trauma centers throughout the United States. Only preliminary results from the trial have been reported by Northfield Laboratories in the press:[51] mortality at 30 days was not statistically different in control patients compared with those treated with PolyHeme (9.6 versus 13.4%). The results of these studies should be considered in the context of bleeding patients for whom early access to blood transfusion is not possible. Even in the United States, almost 50 million Americans live more than an hour away from a trauma center where blood is available. Mortality rates in such a scenario could be considerably higher than those observed in the control patients in the urban setting of the studies discussed earlier, for whom transit times were relatively short. If these data are extrapolated to patients who need an oxygen carrier and have delayed access to blood, HBOCs such as PolyHeme could play an important role in saving lives.

Perioperative Blood Losses

Given the concerns about transfusion-related illness and the general fear of transfusions by the public and physicians, it is common for patients to ask for alternatives to

banked blood. Almost all of the paradigms and algorithms for avoiding transfusion tend to be resource intense and expensive, and still do not eliminate all risk. Alternatives can be sought, and the use of HBOCs as part of intraoperative autologous donation makes a great deal of sense. HBOCs may also be valuable during the perioperative period when blood is often transfused in small quantities to maximize the patient's oxygen-carrying capacity and to replace normal expected surgical blood loss. It is clear that blood substitutes will never be able to completely replace entire circulating blood volume. They might, therefore, be considered more a ''bridge'' until blood transfusion is available or be used to avoid RBC transfusion altogether when blood losses are moderate. Cross-linked[26,52] and polymerized hemoglobin[27–29,53,54] have shown efficacy in several studies (in cardiac[26–28,53,54] and noncardiac[29,52] surgery) that used blood sparing in the perioperative period as the efficacy end point. These studies showed a subsequent reduction in RBC transfusions at 24 hours; however, due to the short half-life of these products, this effect was transient. The adverse effects encountered in these different studies were transient increases in blood pressure, jaundice, and transient, mild increases in enzyme levels that were not obviously related to pathologic liver dysfunction or pancreatitis. Nevertheless, one of these studies was terminated early because of these safety concerns.[52] In two small studies of patients before[55] and after[56] repair of abdominal aortic aneurysm, DCLHb was associated with a decrease in cardiac output due to an increase in afterload, which may pose a serious problem in patients who have altered left ventricular function. Of note, one patient treated with DCLHb had a myocardial infarction 36 hours post infusion.[56] Olofsson and colleagues[57] conducted a safety phase II study in patients undergoing major orthopedic study. These investigators compared Ringer's acetate with two different doses of Hemospan (Sangart, Inc., San Diego, California) given before the induction of anesthesia. Hemospan mildly elevated hepatic enzymes and lipase and was associated with less hypotension and more bradycardic events. A randomized controlled, multicenter phase III trial using a polymerized bovine hemoglobin (Hemopure; Biopure Corporation, Cambridge, Massachusetts) in elective orthopedic surgery was completed in the United States in which 350 patients received Hemopure and 338 received RBC transfusions. The investigators reported that 59.4% of the patients receiving Hemopure were able to avoid allogeneic RBC transfusions; mortality and serious adverse events were comparable in the two treatment groups.[58]

Polymerized bovine hemoglobin (Hemopure) received regulatory approval in South Africa in 2001 for clinical use in the treatment of acute anemia and the avoidance of allogeneic blood transfusion during surgery in adults. Nevertheless, a recent meta-analysis[59] showed that the administration of HBOCs in patients who had surgery, trauma, or stroke was associated with a significant risk of myocardial infarction and death. The meta-analysis of 16 clinical trials with five very different HBOCs showed that administration of any of the products was associated with a higher relative risk of death and of heart attack than was observed in the control groups in the trials; however, the limitation of this analysis was the pooling of very different products used in different settings against different comparators. Some of these products have been withdrawn from development and some of the respective companies have since gone out of business.

Another potential benefit of HBOCs would be in the patient who has multiple antibodies to RBC surface antigens because of multiple previous transfusions and who risks serious transfusion reactions if further RBC transfusion were required. Finally, the recombinant technology could theoretically offer a new therapeutic option in the management of Jehovah's Witness patients.

USE OF HEMOGLOBIN-BASED OXYGEN CARRIERS AS "HEMOGLOBIN THERAPEUTICS"

Sepsis and Septic Shock

Sepsis is characterized by profound microcirculatory alterations.[3,60] With their potentially favorable rheologic properties, HBOCs may increase tissue oxygen availability and oxygen extraction capacity. In septic rats, Sielenkämper and colleagues[61] demonstrated a beneficial effect of DCLHb on tissue oxygen use. When oxygen supply dependency was induced by progressive hemodilution, DCLHb infusion increased oxygen uptake and reversed lactic acidosis. The authors also studied the effects of DCLHb on blood flow distribution and tissue oxygen extraction capabilities in a canine model of endotoxic shock.[62] DCLHb had a dose-dependent vasopressor effect but did not significantly alter cardiac index or regional blood flow. The administration of DCLHb was also followed by an improvement in whole-body oxygen-extraction capabilities.

During sepsis, an increase in inducible NO synthase (NOS) activation leads to overproduction of NO, which is thought to play a role in the pathogenesis of associated hypotension.[63–65] Blockers of NOS activity in animal and clinical studies attenuate these pressure-related effects;[66] however, excessive NO blockade can be harmful,[67] and the use of HBOCs may provide an alternative means of restoring blood pressure and oxygen-extraction capabilities by scavenging NO rather than by entirely blocking NO production. Infusion of pyridoxylated hemoglobin polyoxyethylene (PHP) conjugate in endotoxemic sheep has been shown to have less effect on cardiac index and pulmonary vascular resistance than NOS inhibition with NG-nitro-L-arginine methyl ester.[68] In other studies, HBOC solutions showed protective effects on the kidney by the combined effects of volume expansion and inactivation of NO.[17,69,70] In experimental sepsis, HBOCs have also been shown to prevent myocardial dysfunction.[71,72] In summary, compared with harmful total NO blockade, the use of HBOCs to scavenge NO may provide an alternative means of restoring blood pressure and oxygen-extraction capabilities without entirely blocking NO activity. In a small preliminary study of 14 patients in septic shock, DCLHb infusion allowed a reduction in vasopressor needs to support systemic blood pressure.[25] Nevertheless, this vasopressor effect was accompanied by a reduction in cardiac index and oxygen delivery.[25] A transient increase in total plasma bilirubin was also observed in patients treated with DCLHb.[25]

Recently, Apex Bioscience (Chapel Hill, North Carolina) announced the termination of a study assessing the safety and efficacy of PHP in patients who have distributive shock. This study was a placebo-controlled, randomized, open label study at 15 sites that was initially designed to enroll 1000 patients. The study was terminated early, at 62 patients, because of a protocol design issue (about 800 screen failures due to the absence of a pulmonary artery catheter in situ, an inclusion criterion for the study). Recently published results[73] noted that overall 28-day mortality was similar in the group of patients treated with PHP and in the control group. Nevertheless, survivors treated with PHP tended to be weaned off vasopressors faster and to spend less time on mechanical ventilation.

Although HBOCs may provide an alternative means of restoring blood pressure, improving cellular oxygen availability, and scavenging excess NO release, the potentially deleterious immunomodulatory effects of such solutions in sepsis must be considered. The preparation of these solutions is safe with respect to viral transmission, but because hemoglobin provides an important source of iron, which is necessary to sustain rapid multiplication and growth of bacteria, concerns have been raised about the possibility that hemoglobin solutions could promote infection.[74] Free hemoglobin can also abolish the bactericidal and bacteriostatic effects of plasma

and inactivate neutrophils.[75] Griffiths and colleagues[76] reported that after sublethal *Escherichia coli* challenge in an experimental peritonitis model in mice, survival was reduced after a large intravenous dose of modified hemoglobin solution. Su and colleagues[77] reported that αα-Hb intensified the tumor necrosis factor response to lipopolysaccharide and that mortality after lipopolysaccharide injection was increased in αα-Hb–pretreated mice compared with control mice. The metabolism of these modified hemoglobins may also pose a problem, because their entrapment by macrophages may result in rapid saturation of the reticuloendothelial system, leading to a potential immunodepressant effect. Finally, free iron derived from hemoglobin breakdown can catalyze the production of oxygen free radicals, and the uptake of hemoglobin or ferruginous debris by macrophages may amplify the proinflammatory response, thereby increasing the release of cytokines such as tumor necrosis factor.[78–80] Liposome encapsulation may prevent this phenomenon.[81,82] Promising effects of HBOCs on oxygen transport and the microcirculation in sepsis need to be confirmed, and results from continuing research are eagerly awaited.

Stroke and Myocardial Infarction

Despite promising results in experimental models,[83–85] DCLHb has not been shown to be safe in patients who have cerebral infarction. Saxena and colleagues[86] assessed the safety and tolerability of DCLHb started within 18 hours of symptom onset in patients who had acute ischemic stroke. DCLHb caused a rapid rise in mean arterial pressure. Two patients infused with the high dose of DCLHb (100 mg/kg) had adverse events that were possibly drug related: one suffered fatal brain and pulmonary edema, the other experienced transient renal and pancreatic insufficiency. Multivariate logistic regression analysis showed that DCLHb treatment was an independent predictor of a worse outcome (odds ratio 4.0; confidence interval: 1.4–12.0).

Several experimental studies have demonstrated that HBOCs could preserve myocardial function and reduce infarct size after acute myocardial ischemia[87] by reducing ischemia-reperfusion injury.[88,89]

Sickle Cell Anemia

Patients who have sickle cell anemia comprise a unique population that might receive added benefits from HBOCs. These patients tend to be chronically transfused and also seem to be at a higher risk for becoming alloimmunized per unit transfused compared with the general population. The universal compatibility of HBOCs would be ideal to protect these patients from further alloimmunization and the risks of delayed hemolytic transfusion reactions. In addition, the availability of blood for sickle cell patients requiring phenotyped RBCs would be improved. HBOCs could be useful as urgent therapy for acute chest syndrome or other emergency vaso-occlusive events. These solutions may be useful to bridge patients who are alloimmunized until compatible blood can be found. There are a number of case reports in the literature of sickle cell patients who have benefited from HBOCs.[90–92] Although results from case reports are dramatic, larger studies with appropriate controls are required to verify these intriguing findings.

SUMMARY

Hemoglobin solutions were initially developed with the hope of finding an alternative to the problems associated with blood transfusion; however, with the realization that hemoglobin solutions not only are RBC substitutes but also have a number of additional properties, a new, broader concept of hemoglobin therapeutics developed.

Promising effects on oxygen transport and the microcirculation need to be confirmed, and the results of continuing research are eagerly awaited. Although debatable, the results of a recent meta-analysis[59] showing that the administration of HBOCs in surgical, stroke, and trauma patients was associated with a significant risk of myocardial infarction and death remind us that no drug is perfect or without side effects, and HBOCs are no exception. Today, we know the effects of the first generation of HBOCs. One area of interest is the development of new molecular structures to decrease NO binding so as to minimize the adverse consequences and to maximize the potential benefits. It is also fascinating to speculate that the choice among the various available solutions, with their different oxygen affinities, oxygen-carrying capacities, viscosities, and oncotic pressures, may be guided by clinical circumstances. Nevertheless, possible adverse effects need to be carefully evaluated before these agents can be widely administered in the ICU, emergency room, or prehospital setting.

REFERENCES

1. Winslow RM. Blood substitutes: refocusing an elusive goal. Br J Haematol 2000; 111:387–96.
2. Wagner PD. An integrated view of the determinants of maximum oxygen uptake. In: Gonzales NC, Fredde MR, editors. Oxygen transfer from atmosphere to tissues. New York: Plenum; 1988. p. 245–56.
3. De Backer D, Creteur J, Preiser JC, et al. Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med 2002;166:98–104.
4. Vlahakes GJ, Lee R, Jacobs EE, et al. Hemodynamic effects and oxygen transport properties of a new blood substitute in a model of massive blood replacement. J Thorac Cardiovasc Surg 1990;100:379–88.
5. Przybelski RJ, Malcolm DS, Burris DG, et al. Cross-linked hemoglobin solution as a resuscitative fluid after hemorrhage in the rat. J Lab Clin Med 1991;117: 143–51.
6. Cohn SM, Farrell TJ. Diaspirin cross-linked hemoglobin resuscitation of hemorrhage: comparison of a blood substitute with hypertonic saline and isotonic saline. J Trauma 1994;39:210–7.
7. Lee R, Neya K, Svizzero TA, et al. Limitations of the efficacy of hemoglobin-based oxygen-carrying solutions. J Appl Physiol 1995;79:236–42.
8. Leppäniemi A, Soltero R, Burris D, et al. Early resuscitation with low-volume poly-DCLHb is effective in the treatment of shock induced by penetrating vascular injury. J Trauma 1996;40:242–8.
9. Hughes GS Jr, Antal EJ, Locker PK, et al. Physiology and pharmacokinetics of a novel hemoglobin-based oxygen carrier in humans. Crit Care Med 1996;24: 756–64.
10. Kerger H, Tsai AG, Saltzman DJ, et al. Fluid resuscitation with O_2 vs. non-O_2 carriers after 2h of hemorrhagic shock in conscious hamsters. Am J Physiol 1997;272:H525–37.
11. Migita R, Gonzales A, Gonzales ML, et al. Blood volume and cardiac index in rats after exchange transfusion with hemoglobin-based oxygen carriers. J Appl Physiol 1997;82:1995–2002.
12. Cole DJ, Drummond JC, Patel PM, et al. Effect of oncotic pressure of diaspirin cross-linked hemoglobin (DCLHb) on brain injury after temporary focal cerebral ischemia in rats. Anesth Analg 1996;83:342–7.

13. Intaglietta M, Johnson PC, Winslow RM. Microvascular and tissue oxygen distribution. Cardiovasc Res 1996;32:632–43.
14. Kuchan MJ, Frangos JA. Shear stress regulates endothelin-1 release via protein kinase C and cGMP in cultured endothelial cells. Am J Physiol 1993;264: H150–6.
15. Kuchan MJ, Jo H, Frangos JA. Role of G proteins in shear stress-mediated nitric oxide production by endothelial cells. Am J Physiol 1994;267:C753–8.
16. Tsai AG, Friesenecker B, McCarthy M, et al. Plasma viscosity regulates capillary perfusion during extreme hemodilution in hamster skinfold model. Am J Physiol 1998;275:H2170–80.
17. Thompson A, McGarry AE, Valeri CR, et al. Stroma-free hemoglobin increases blood pressure and GFR in the hypotensive rat: role of nitric oxide. J Appl Physiol 1994;77:2348–54.
18. Sharma AC, Gulati A. Effect of diaspirin cross-linked hemoglobin and norepinephrine on systemic hemodynamics and regional circulation in rats. J Lab Clin Med 1994;123:299–308.
19. Sharma AC, Singh G, Gulati A. Role of NO mechanism in cardiovascular effects of diaspirin cross-linked hemoglobin in anesthetized rats. Am J Physiol 1995;269: H1379–88.
20. Hindman BJ, Dexter F, Cutkomp J, et al. Diaspirin cross-linked hemoglobin dos not increase brain oxygen consumption during hypothermic cardiopulmonary bypass in rabbits. Anesthesiology 1995;83:1302–11.
21. Aranow JS, Wang H, Zhuang J, et al. Effect of human hemoglobin on systemic and regional hemodynamics in a porcine model of endotoxemic shock. Crit Care Med 1996;24:807–14.
22. Gulati A, Sharma AC, Singh G. Role of endothelin in the cardiovascular effects of diaspirin crosslinked and stroma reduced hemoglobin. Crit Care Med 1996;24: 137–47.
23. Kasper SM, Grune F, Walter M, et al. The effects of increased doses of bovine hemoglobin on hemodynamics and oxygen transport in patients undergoing preoperative hemodilution for elective abdominal aortic surgery. Anesth Analg 1998;87:284–91.
24. Przybelski RJ, Daily EK, Kisicki JC, et al. Phase I study of the safety and pharmacologic effects of diaspirin cross-linked hemoglobin solution. Crit Care Med 1996; 24:1993–2000.
25. Reah G, Bodenham AR, Mallick A, et al. Initial evaluation of diaspirin cross-linked hemoglobin (DCLHb) as a vasopressor in critically ill patients. Crit Care Med 1997;25:1480–8.
26. Lamy ML, Daily EK, Brichant JF, et al. Randomized trial of diaspirin cross-linked hemoglobin solution as an alternative to blood transfusion after cardiac surgery. Anesthesiology 2000;92:646–56.
27. Hill SE, Gottschalk LI, Grichnik K. Safety and preliminary efficacy of hemoglobin raffimer for patients undergoing coronary artery bypass surgery. J Cardiothorac Vasc Anesth 2002;16:695–702.
28. Levy JH, Goodnough LT, Greilich PE, et al. Polymerized bovine hemoglobin solution as a replacement for allogeneic red blood cell transfusion after cardiac surgery: results of a randomized, double-blind trial. J Thorac Cardiovasc Surg 2002;124:35–42.
29. LaMuraglia GM, O'Hara PJ, Baker WH, et al. The reduction of the allogenic transfusion requirement in aortic surgery with a hemoglobin-based solution. J Vasc Surg 2000;31:299–308.

30. Rooney MW, Hirsch LJ, Mathru M. Hemodilution with oxyhemoglobin: mechanism of oxygen deivery and its superaugmentation with a nitric oxide donor (sodium nitroprusside). Anesthesiology 1993;79:60–72.
31. Schultz SC, Grady B, Cole F, et al. A role for endothelin and nitric oxide in the pressor response to diaspirin cross-linked hemoglobin. J Lab Clin Med 1993; 122:301–8.
32. Freas W, Llave R, Jing M, et al. Contractile effects of diaspirin cross-linked hemoglobin (DCLHb) on isolated porcine blood vessels. J Lab Clin Med 1995;125: 762–7.
33. Ulatowski JA, Nishikawa T, Matheson-Urbaitis B, et al. Regional blood flow alterations after bovine fumaryl ββ-crosslinked hemoglobin transfusion and nitric oxide synthase inhibition. Crit Care Med 1996;24:558–65.
34. Hart JL, Ledvina MA, Muldoon SM. Actions of diaspirin cross-linked hemoglobin on isolated rat and dog vessels. J Lab Clin Med 1997;129:356–63.
35. Resta TC, Walker BR, Eichinger MR, et al. Rate of NO scavenging alters effects of recombinant hemoglobin solutions on pulmonary vasoreactivity. J Appl Physiol 2002;93:1327–36.
36. Rudolph AS, Sulpizio A, Hieble P, et al. Liposome encapsulation attenuates hemoglobin-induced vasoconstriction in rabbit arterial segments. J Appl Physiol 1997;82:1826–35.
37. Bone HG, Schenarts PJ, Booke M, et al. Oxalated pyridoxalated hemoglobin polyoxyethylene conjugate normalizes the hyperdynamic circulation in septic sheep. Crit Care Med 1997;25:1010–8.
38. Gulati A, Singh R, Chung SM, et al. Role of endothelin-converting enzyme in the systemic hemodynamics and regional circulatory effects of proendothelin-1 (1–38) and diaspirin cross-linked hemoglobin in rats. J Lab Clin Med 1995;126:559–70.
39. Rohlfs RJ, Bruner E, Chiu A, et al. Arterial blood pressure responses to cell-free hemoglobin solutions and the reaction with nitric oxide. J Biol Chem 1998;273: 12128–34.
40. Sakai H, Hara H, Yuasa M, et al. Molecular dimensions of Hb-based O(2) carriers determine constriction of resistance arteries and hypertension. Am J Physiol Heart Circ Physiol 2000;279:H908–15.
41. Bouwer ST, Hoofd L, Kreuzer F. Diffusion coefficients of oxygen and hemoglobin measured by facilitated oxygen diffusion through hemoglobin solutions. Biochim Biophys Acta 1997;1338:127–36.
42. Nishide H, Chen XS, Tsuchida E. Facilitated oxygen transport with modified and encapsulated hemoglobins across non-flowing solution membrane. Artif Cells Blood Substit Immobil Biotechnol 1997;25:335–46.
43. Lindbom L, Tuma RF, Arfors KE. Influence of oxygen on perfused capillary density and capillary red cell velocity in rabbit skeletal muscle. Microvasc Res 1980;19: 197–208.
44. McCarthy MR, Vandegriff KD, Winslow RM. The role of facilitated diffusion in oxygen transport by cell-free hemoglobins: implications for the design of hemoglobin-based oxygen carriers. Biophys Chem 2001;92:103–17.
45. Winslow RM, Gonzales A, Gonzales ML, et al. Vascular resistance and the efficacy of red cell substitutes in a rat hemorrhage model. J Appl Physiol 1998;85: 993–1003.
46. Wettstein R, Tsai AG, Erni D, et al. Resuscitation with polyethylene glycol-modified human hemoglobin improves microcirculatory blood flow and tissue oxygenation after hemorrhagic shock in awake hamsters. Crit Care Med 2003; 31:1824–30.

47. Gould SA, Moore EE, Hoyt DB, et al. The first randomized trial of human polymerized hemoglobin as a blood substitute in acute trauma and emergent surgery. J Am Coll Surg 1998;187:113–20.
48. Gould SA, Moore EE, Hoyt DB, et al. The life-sustaining capacity of human polymerized hemoglobin when red cells might be unavailable. J Am Coll Surg 2002; 195:445–52.
49. Sloan EP, Koenigsberg M, Gens D, et al. Diaspirin cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock: a randomized controlled efficacy trial. JAMA 1999;282:1857–64.
50. Kerner T, Ahlers O, Veit S, et al. DCL-Hb for trauma patients with severe hemorrhagic shock: the European On-Scene multicenter study. Intensive Care Med 2003;29:378–85.
51. Northfield laboratories reports results of pivotal phase III trauma study [news release]. Evanston (IL): Northfield Laboratories, Inc; 2007. Available at: http://phx.corporate-ir.net/phoenix.zhtml?c=91374&p=irol-newsArticle_print&ID=1005951&highlight=. Accessed June 5, 2008.
52. Schubert A, Przybelski R, Eidt J, et al. Diaspirin-crosslinked haemoglobin reduces blood transfusion in non cardiac surgery: a multicenter, randomized, controlled, double-blinded trial. Anesth Analg 2003;97:323–32.
53. Cheng D, Mazer C, MArtineau R, et al. A phase II dose-response study of hemoglobin raffimer (Hemolink) in elective coronary artery bypass surgery. J Thorac Cardiovasc Surg 2004;127:79–86.
54. Greenburg A, Kim H, Hemolink Study Group. Use of an oxygen therapeutic as an adjunct to intraoperative autologous donation to reduce transfusion requirements in patients undergoing coronary artery bypass surgery. J Am Coll Surg 2004;198: 373–83.
55. Garrioch M, McClure J, Wildsmith J. Haemodynamic effects of diaspirin cross-linked haemoglobin (DCLHb) given before abdominal aortic aneurysm surgery. Br J Anaesth 1999;83:702–7.
56. Bloomfield E, Rady M, Esfandiari S. A prospective trial of diaspirin-linked hemoglobin solution in patients after elective repair of abdominal aortic aneurysm. Mil Med 2004;169:546–50.
57. Olofsson C, Ahl T, Johansson T, et al. A multicenter clinical study of the safety and activity of maleimide-polyethylene glycol-modified Hemoglobin (Hemospan) in patients undergoing major orthopaedic surgery. Anesthesiology 2006;105: 1153–63.
58. Jahr JS, Mackenzie C, Pearce LB, et al. vHBOC-201 as an alternative to blood transfusion: efficacy and safety evaluation in a multicenter phase III trial in elective orthopedic surgery. J Trauma 2008;64:1484–97.
59. Natanson C, Kern S, Lurie P, et al. Cell-free hemoglobin-based blood substitutes and risk of myocardoial infarction and death: a meta-analysis. JAMA 2008;299: 2304–12.
60. Lam C, Tyml K, Martin C, et al. Microvascular perfusion is impaired in a rat model of normotensive sepsis. J Clin Invest 1994;94:2077–83.
61. Sielenkämper AW, Chin-Yee IH, Martin C, et al. Diaspirin crosslinked hemoglobin improves systemic oxygen uptake in oxygen-supply dependent septic rats. Am J Respir Crit Care Med 1997;156:1066–72.
62. Creteur J, Zhang H, De Backer D, et al. Diaspirin cross-linked hemoglobin improves oxygen extraction capabilities in endotoxic shock. J Appl Physiol 2000;89:1437–44.

63. Wong HR, Carcillo JA, Burckart G, et al. Increased serum nitrite and nitrate concentrations in children with the sepsis syndrome. Crit Care Med 1995;23: 835–42.
64. Gomez-Jimenez J, Salgado A, Mourelle M, et al. L-arginine: nitric oxide pathway in endotoxemia and human septic shock. Crit Care Med 1995;23:253–8.
65. Szabo C. Alterations in nitric oxide production in various forms of circulatory shock. New Horiz 1995;3:2–32.
66. Vincent JL, Preiser JC, Zhang H. Blocking the effects of nitric oxide in septic shock. In: Fink MP, Payen D, editors, Role of nitric oxide in septic shock—update in intensive care and emergency medicine, Vol 24. Berlin: Springer-Verlag; 1995. p. 253–73.
67. Lopez A, Lorente JA, Steingrub J, et al. Multiple-center, randomized, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock. Crit Care Med 2004;32:21–30.
68. Bone HG, Waurick R, Van Aken H, et al. Comparison of the hemodynamic effects of nitric oxide synthase inhibition and nitric oxide scavenging in endotoxemic sheep. Intensive Care Med 1998;24:48–54.
69. Matheson-Urbaitis B, Lu YS, Fronticelli C, et al. Renal and systemic-hemodynamic response to isovolemic exchange transfusion with hemoglobin cross-linked with bis (3, 5-dibromosalicyl) fumarate or albumin. J Lab Clin Med 1995; 126:250–60.
70. Heneka MT, Löschmann PA, Osswald H. Polymerized hemoglobin restores cardiovascular and kidney function in endotoxin-induced shock in the rat. J Clin Invest 1997;99:47–54.
71. Neviere R, Sielenkamper A, Pitt M, et al. Diaspirin crosslinked hemoglobin prevents endotoxin induced myocardial contractile dysfunction in an isolated and perfused rat heart model. Am J Respir Crit Care Med 1997;155:A926 [abstract].
72. Cooper A, Ellis C, Lin HY, et al. Effect of diaspirin crosslinked hemoglobin (DCLHB) treatment on myocardial blood flow heterogeneity in septic sheep. Am J Respir Crit Care Med 1997;155:A926 [abstract].
73. Kinasewitz GT, Imm A, Steingrub JS, et al. Multi-center, randomized, placebo-controlled study of the nitric oxide scavenger pyridoxalated hemoglobin polyoxyethylene in distributive shock. Crit Care Med 2008;36:1999–2007.
74. Lee JT, Arenholz DH, Nelson RD, et al. Mechanisms of the adjuvant effect of hemoglobin in experimental peritonitis V: the significance of the coordinated iron component. Surgery 1979;86:41–7.
75. Kim YM, Yamazaki I, Piette LH. The effect of haemoglobin, hematin and iron on neutrophil inactivation in superoxide generating systems. Arch Biochem Biophys 1994;309:308–14.
76. Griffiths E, Cortes A, Gilbert N, et al. Haemoglobin-based blood substitutes and sepsis. Lancet 1995;345:158–60.
77. Su D, Roth RI, Levin J. Hemoglobin infusion augments the tumor necrosis factor response to bacterial endotoxin (lipopolysaccharide) in mice. Crit Care Med 1999;27:771–8.
78. Sherry BA, Alava G, Tracey KJ, et al. Malaria-specific metabolite hemozoin mediates the release of several potent endogenous pyrogens (TNF, MIP-1α and MIP-1β) in vitro, and altered thermoregulation in vivo. J Inflamm 1995;45:85–96.
79. McFaul SJ, Bowman PD, Villa VM, et al. Hemoglobin stimulates mononuclear cells to release interleukin-8 and tumor necrosis factor α. Blood 1994;84:3175–81.

80. Carrillo EH, Gordon LE, Richardson JD, et al. Free hemoglobin enhances tumor necrosis factor-alpha production in isolated human monocytes. J Trauma 2002; 52:449–52.
81. Rabinovici R, Rudolph AS, Vernick J, et al. Lyophilized liposome encapsulated hemoglobin: evaluation of hemodynamic, biochemical, and hematologic responses. Crit Care Med 1994;22:480–5.
82. Rudolph AS, Cliff R, Kwasiborski V, et al. Liposome-encapsulated hemoglobin modulates lipopolysaccharide-induced tumor necrosis factor-α production in mice. Crit Care Med 1997;25:460–8.
83. Chappell JE, McBride WJ, Shackford SR. Diaspirin cross-linked hemoglobin resuscitation improves cerebral perfusion after head injury and shock. J Trauma 1996;41:781–8.
84. Bowes MP, Burhop KE, Zivin JA. Diaspirin cross-linked hemoglobin improves neurological outcome following reversible but not irreversible CNS ischemia in rabbits. Stroke 1994;25:2253–7.
85. Cole DJ, Schell RM, Drummond JC, et al. Focal cerebral ischemia in rats: effect of hemodilution with α-α cross-linked hemoglobin on brain injury. Can J Neurol Sci 1993;20:30–6.
86. Saxena R, Wijnhoud AD, Carton H, et al. Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb, in acute ischemic stroke. Stroke 1999;30:993–6.
87. George I, Yi G, Schulman A, et al. A polymerized bovine haemoglobin oxygen carrier preserves regional myocardial function and reduces infarct size after acute myocardial ischemia. Am J Physiol Heart Circ Physiol 2006;291:H1126–37.
88. Burmeister M, Rempf C, Standl T, et al. Effect of prophylactic or therapeutic application of bovine haemoglobin HBOC-200 on ischaemia-reperfusion injury following acute coronary ligature in rats. Br J Anaesth 2005;95:737–45.
89. Caswell J, Strange M, Rimmer D, et al. A novel hemoglobin-based blood substitute protects against myocardial reperfusion injury. Am J Physiol Heart Circ Physiol 2005;288:H1796–801.
90. Raff JP, Dobson CE, Tsai H. Transfusion of polymerized human haemoglobin in a patient with severe sickle-cell anaemia. Lancet 2002;60:464–5.
91. Lanzkron S, Moliterno AR, Norris EJ, et al. Polymerized human Hb use in acute chest syndrome: a case report. Transfusion 2002;42:1422–6.
92. Gonzalez P, Hackney AC, Jones S, et al. A phase I/II study of polymerized bovine hemoglobin in adult patients with sickle. J Investig Med 1997;45:258–64.