Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Transfusion clinique et biologique 12 (2005) 374–379
TRANSFUSION CLINIQUE ET BIOLOGIQUE
http://france.elsevier.com/direct/TRACLI/

EuroSAT 2005

What happened to blood substitutes?

Qu'est il arrivé aux substituts du sang ?

C.P. Stowell

Blood Transfusion Service, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street, Boston, MA 02114-2696, USA

Abstract

Concerns about the safety and adequacy of the blood supply have fostered twenty years of research into the so-called "blood substitutes" among them the oxygen carriers based on modified hemoglobin. Although none of these materials has yet been licensed for use in North America or Europe, the results of research and clinical trials have increased our understanding of oxygen delivery and its regulation. In particular, the examination of the basis for the vasoactivity observed with some of the hemoglobin based oxygen carriers has led to the insight that several colligative properties of hemoglobin solutions, such as their diffusion coefficient for oxygen, viscosity and colloid oncotic pressure, are important determinants of efficacy.

Keywords: Blood substitutes; Transfusion; Hemoglobin based oxygen carriers; Oxygen delivery

1. Introduction

The two major impulses driving the development of blood substitutes are concerns about the infectious risks of transfusion and the adequacy of the blood supply. Although the incidence of transfusion transmitted HIV, hepatitis B virus and hepatitis C virus has been greatly reduced since the mid-1980s, the threat of new or emerging pathogens, such as West Nile virus and the corona virus responsible for severe acute respiratory syndrome, continue to motivate research into an oxygen carrier which is free of infectious agents. Furthermore, episodic blood shortages and the gap between the growing transfusion needs of an aging population expecting access to increasingly sophisticated medical care, and the shrinking proportion of the public who are willing and able to donate blood, make a synthetic or semi-synthetic oxygen carrier a desirable adjunct to banked blood.

Three major classes of materials have been studied as blood substitutes: perfluorocarbon emulsions, modified hemoglobin solutions, and liposome-enclosed hemoglobin. Only the perfluorocarbon emulsions and the modified hemoglobin solutions have reached the level of clinical trials; liposome-enclosed hemoglobin remains in the pre-clinical stage of testing. A number of reviews have discussed various aspects of blood substitutes [1–4]. This review will focus on the hemoglobin-based oxygen carriers (HBOC).

Hemoglobin is an obvious candidate as a blood substitute with a number of desirable characteristics. It has a high capacity for O_2; it lacks the numerous and complex antigens of the red blood cell membrane, hence it is universally compatible; it is a robust molecule which withstands rigorous purification and viral inactivation processes; it is stable under ordinary storage conditions; and its physiology was thought to be well understood, although time has shown that there is more to be learned. Thus, eight different companies embarked on the development of a HBOC in the 1980s and 1990s (see Table 1). To date, one product, Oxyglobin, has been licensed for veterinary use, while its sister product, Hemopure, has been approved for limited use in humans in South Africa. However,

E-mail address: cstowell@partners.org (C.P. Stowell).

1246-7820/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.tracli.2005.10.002

Table 1
Hemoglobin based oxygen carriers in clinical trials[a]

Product (manufacturer)	*Hemoglobin source* (Modification)	*Clinical trial level*	*Application*
PHP (Ajinomoto/Apex)	*Human* (PEG conjugated)	Phase III (discontinued)	NO induced shock
HemAssist (Baxter)	*Human* (cross-linked)	Phase II Phase III (discontinued)	Septic shock, hemodialysis, hemorrhagic shock, cardio-pulmonary bypass Acute blood loss-surgery, trauma Stroke
Optro (Somatogen/Baxter)	*Recombinant* (cross-linked)	Phase II Phase I (discontinued)	ANH, surgery Erythropoiesis in ESRD, refractory anemia
Hemopure (Biopure)	*Bovine* (polymerized)	Preclinical Phase I Phase II Phase III[b]	Erythropoiesis Radiosensitizer, glioblastoma Sickle cell crisis, oncology, surgery-orthopedic, urological, vascular, cardiac, trauma, cardioprotectant PTCA Surgery-cardiac, orthopedic
Oxyglobin (Biopure)	*Bovine* (polymerized)	Approved	Veterinary-anemia, acute blood loss
PEG hemoglobin (Enzon)	*Bovine* (PEG conjugated)	Phase Ib (discontinued)	radiosensitizer solid tumors
Hemolink (Hemosol)	*Human* (polymerized)	Phase II Phase III (discontinued)	Cardiopulmonary bypass- ANH, orthopedic surgery acute blood loss, dialysis Cardiac surgery
PolyHeme (Northfield)	*Human* (polymerized)	Phase III	Trauma, surgery
Hemospan (Sangart)	*Human* (PEG conjugated)	Phase II	Surgery

ANH: acute normovolemic hemodilution; ESRD: end-stage renal disease; PTCA: percutaneous transluminal coronary angioplasty.
[a] Information current to 9/05.
[b] Approved in South Africa.

only three companies are still actively engaged in clinical trials of HBOCs (Northfield, Biopure, Sangart).

2. Results of clinical trials

HemAssist—Baxter—HemAssist is a human hemoglobin tetramer stabilized by a di-aspirin linkage [5]. It was in Phase III clinical trials in trauma, surgery and acute ischemic stroke when in 1998 the company halted further development. In both the stroke [6] and the trauma trial [7] excess mortality was observed among the patients receiving HemAssist compared to those receiving banked red blood cells. However, mortality was equivalent among patients receiving HemAssist or banked red blood cells in a trial in cardiac surgery [8]. A post-hoc analysis of the trauma trial failed to identify the reason for the unexpected high mortality rate in the HemAssist treated patients [9]. Subsequently, however, a study was published comparing resuscitation with normal saline or the same di-aspirin cross-linked hemoglobin tetramer as HemAssist in an animal model of traumatic brain injury [10]. The mean arterial pressure was higher and the cardiac output was lower in the hemoglobin-resuscitated animals. In addition, the cerebral O_2 saturation was lower suggesting that O_2 delivery was impaired, perhaps the result of a vasoconstrictive response to the HBOC.

Hemolink–Hemosol–This product consists of human hemoglobin polymerized using an oxidized trisaccharide, O-raffinose, followed by a reduction step [11]. It was studied in Phase II clinical trials in dialysis and as an oxygen carrying replacement fluid in acute normovolemic hemodilution where it was noted to have a mild systemic pressor effect [12]. In Phases II and III studies in cardiac surgery, patients receiving up to 4 units of Hemolink required fewer transfusions of banked red blood cells up to 5 days after surgery compared to controls receiving pentastarch [13,14]. However, the company announced that there were safety concerns in the Phase III study and has since not initiated any new trials with this product [15].

Hemopure–Biopure–Hemopure, and the veterinary formulation, Oxyglobin, consists of bovine hemoglobin which has been polymerized with glutaraldehyde and purified to reduce residual hemoglobin tetramers (< 3%). It has been studied principally for perioperative use as a ‘bridge’, deferring the need for banked red blood cells [16–19]. It has also been noted to have a pressor effect which correlates with increased systemic vascular resistance and decreased cardiac index. In a study of patients undergoing infrarenal aortic aneurysm resection, 27% of patients randomized to receive Hemopure intraoperatively avoided allogeneic transfusion compared to none of the patients receiving banked red blood cells, although the median number of allogeneic units used was not different [17]. Biopure also completed a Phase III study in non-cardiac surgery and submitted the data to the FDA, which requested additional data. Biopure has since proposed a Phase IIb/III clinical trial in trauma, although the study design has not yet been approved by the FDA [20]. Meanwhile, they have begun to explore an-

other use for Hemopure as a cardioprotective agent in patients undergoing coronary artery angioplasty or stent placement and have completed enrollment in a clinical trial in Europe.

PolyHeme–Northfield–This preparation consists of human hemoglobin, which has been pyridoxilated to increase the P_{50}, polymerized with glutaraldehyde, and purified to remove residual tetramers [21]. It is being developed as an alternative to banked red blood cells in surgery and trauma [22–24]. Northfield submitted data from its Phase III trial in trauma to the FDA. They subsequently initiated a new Phase III trial of PolyHeme in pre-hospital trauma resuscitation and have enrolled more than 400 patients out of a planned 720 [25].

Hemospan–Sangart–The newest HBOC in clinical trials is prepared by conjugating polyethylene glycol (PEG) to human hemoglobin [26]. This product has been designed with a low P_{50}, a large molecular diameter, and a high viscosity [27]. Phase I and II trials have been completed in Europe [28] and another Phase II trial has been initiated in the United States [29].

3. What have we learned?

Although HBOCs have been in development for almost 20 years, no product has yet been licensed for human use with the exception of the limited arrangement for Hemopure in South Africa. Nonetheless, considerable progress has been made in developing products which meet many of the criteria for a clinically useful and safe oxygen carrier including: better shelf stability than banked red cells, universal compatibility, useful vascular half-life, absence of infectious agents, avoidance of the known toxicities related to residual stroma, and absence of renal impairment. The HBOCs under development all have vascular half-lives in the 18–24 h range, which is adequate for most acute care applications (i.e. hemorrhage and surgery). Most can be stored at 4 °C or room temperature for 1–2 years and none of them require any form of compatibility testing. All of them have been successfully processed to eliminate the presence of micro-organisms, although there are very few published data on the removal of prions. None of the HBOCs produce the acute renal injury seen when unmodified hemoglobin is present in the vascular space.

However, pre-clinical, and is some cases, clinical testing of the HBOCs have raised other safety concerns related to vasoactivity and cell toxicity, the latter either as a direct effect or one mediated by oxidative products [30]. Some, but not all of the various HBOCs under development have shown a systemic pressor effect [31–33] and in some cases a pulmonary pressor effect as well [31,34,35] usually accompanied by decreased heart rate and cardiac output, an indicator of increased systemic vascular resistance [36]. Although the observed systemic pressor effect of the HBOCs, which is generally mild, is not necessarily deleterious per se, the possibility that it reflects vasoconstriction is of concern particularly if it prevents effective perfusion of capillary beds and eliminates the benefit of increased blood pressure or increased O_2 carrying capacity. HBOCs with systemic pressor effects have been shown to produce vasoconstriction in animal model systems [37].

The understanding of the mechanisms whereby some HBOCs exert a pressor effect has progressed considerably in the past decade. The rapid binding of nitric oxide (NO) to both oxy- and deoxyhemoglobin [38] and the ability of the HBOCs, which are very small compared to intact erythrocytes, to move in the bloodstream into the RBC free zone close to the vessel wall [39], suggested that they may trigger vasoconstriction by scavenging the NO produced by the vascular endothelium thereby releasing its constitutive vasodilatory influence [40–42]. It was predicted that HBOCs with large molecular weights, which would not be able to extravasate into the subendothelial space very readily, would exert less of vasoconstrictive effect. However, the correlation of pressor effect and molecular weight is weak. Although a substantial pressor effect was seen with stabilized hemoglobin tetramers, such as the diaspirin linked hemoglobin (HemAssist), it was also present in formulations consisting almost entirely of higher order n-mers of hemoglobin with very little residual tetramer, such as PolyHeme and Hemopure [43–45]. The pressor effect also does not correlate well with NO affinity [46]. Therefore, NO scavenging does not seem to be the major mechanism whereby HBOCs exert a vasoconstrictive effect [47].

Other properties of the HBOCs are emerging as important determinants of their ability to deliver oxygen to tissues, among them viscosity. Hemoglobin solutions are much less viscous than whole blood and insofar as the dilution of the circulating blood with an HBOC would lower its viscosity and systemic vascular resistance, it might be expected to improve flow, at least at a systemic level. However, events at the level of the microcirculation may not necessarily reflect systemic hemodynamics [48]. The endothelial cells lining small vessels appear to sense shear stress, a property of a moving fluid, which is directly proportional to viscosity [49]. A drop in shear stress (viscosity) triggers down-regulation of the production of NO by endothelial cells triggering vasoconstriction [50,51]. This viscosity-dependent regulation of flow in the microcirculation has been demonstrated in several experimental systems [52–57].

Shear stress is affected not only by blood viscosity, but by colloid oncotic pressure (COP) as well:

$$\text{Shear stress} = \frac{4\mu Q}{\pi(D/2)^3}$$

where μ = viscosity, Q = net vascular fluid movement which is a function of COP, and D = blood vessel diameter. HBOCs with high COP and high viscosity would be expected to maintain a high level of shear stress and a vasodilated state. In addition, HBOCs with high COP would be expected to maintain intravascular volume and cardiac output, contributing to the maintenance of normovolemia at a systemic level [58] and perhaps in the microcirculation as well, by maintaining shear stress, even in the face of hemodilution [59,60]. When normalized for hemoglobin concentration, HBOCs consisting of polymerized hemoglobin tetramers have lower COP than those con-

sisting of stabilized hemoglobin tetramers or those which have been surface conjugated, and might not be as effective for maintaining intravascular volume [60], or flow though the microcirculation.

Based on the observation that terminal arterioles are innervated, Guyton [61] originally proposed that they may play an active role in regulating blood flow through the capillary beds they supply. In recent years, a more detailed autoregulatory theory has been proposed based on observations in animal experiments and model systems [26]. This theory posits that terminal arterioles respond to local PO_2 by matching flow to the perceived need. Paradoxically, excessive delivery of O_2 at the level of the arteriole might be expected to trigger vasoconstriction, thereby impeding flow and oxygen delivery to the distal capillary beds. Oxygen delivery to the arteriole may be affected by the O_2 content of the blood (which in turn depends on hemoglobin concentration and its degree of O_2 saturation), the ability of hemoglobin to off-load O_2 (determined in part by the P_{50} and Hill coefficient) and the ability of O_2 to diffuse from the red cell, or oxygen carrier, to the vascular endothelium.

The autoregulatory theory is supported by several key observations. The progressive drop in PO_2 as blood flows along the arterial tree and into the capillary bed is well recognized [62]. However, the loss of O_2 is particularly marked at the level of the vasoactive terminal arterioles, where the PO_2 is generally approximately 20–30 mmHg, corresponding to the steep portion of oxy-hemoglobin dissociation curve [63].

In addition, extensive experimentation in animal and model systems has shown that HBOCs which unload O_2 at the level of the pre-capillary arteriole trigger vasoconstriction consistent with this autoregulatory model [64–66]. Several characteristics of the HBOCs may affect their propensity to deliver O_2 to the arterial wall. The presence of hemoglobin in solution is known to enhance the diffusion of O_2 as well as its uptake and release [67–69]. HBOCs are distributed in the red cell free layer of the plasma close to the endothelium, shortening the diffusion path for off-loaded O_2, as well as facilitating diffusion of O_2 from red blood cells through the plasma toward the endothelium. Since the diffusion coefficient of a molecule is inversely related to its molecular radius, a molecule with a small radius, such as a stabilized hemoglobin tetramer, would have a higher diffusion coefficient for O_2 than a hemoglobin conjugated to PEG which complexes with water and sweeps a much larger radius. HBOCs with high diffusion coefficients might be expected to deliver O_2 to the arterial wall more readily. Accordingly, HBOCs with smaller molecular radii, and presumably higher diffusion coefficients for O_2, have been shown to produce vasoconstriction and limit blood flow to distal capillary beds in several experimental systems [70–72].

Another factor which could affect O_2 delivery to the arterial wall is the oxygen affinity of the HBOC. It might be expected that an HBOC with high oxygen affinity (low P_{50}) would unload less O_2 than one with low affinity and therefore be less likely to trigger a vasoconstrictive response [46,64]. In one model system, vasoactivity was found to be greater in an HBOC with a higher P_{50} than a similar preparation with a low P_{50} [46]. However, experiments in an artificial capillary system [70] and animals [71,72] indicate that the diffusion properties of an HBOC make more of a contribution to its vasoactivity than the P_{50}. Hence, the P_{50} seems to play only a secondary role in determining the vasoactivity of an HBOC.

These studies now suggest that an HBOC with high viscosity, high COP and large molecular radius (low O_2 diffusion coefficient) is less likely to trigger a vasoconstrictive response, improving flow and oxygen delivery to the capillary beds. Some of the deleterious effects noted in clinical trials, including the systemic pressor effect, may have reflected regional vasoconstriction and impairment of tissue oxygenation.

4. Conclusion

The search for a clinically useful oxygen carrier has proven to be arduous and time-consuming. However, the studies of the various HBOCs over the past decade have re-shaped our thinking about the mechanisms of oxygen delivery and its regulation. These new insights are paving the way to realizing the goal of adding a blood substitute to the therapeutic armamentarium.

References

[1] Stowell CP, Levin J, Spiess BD, Winslow RM. Progress in the development of blood substitutes. Transfusion 2001;41:287–99.
[2] Stowell CP. Hemoglobin based oxygen carriers. Curr Opin Hematol 2002;9:537–43.
[3] Hess JR. Update on alternative oxygen carriers. Vox Sang 2004;87(Suppl 2):132–5.
[4] Bloomfield EL, Leone BJ. The safety of hemoglobin blood substitutes. Anesth Analg 2003;97:323–32.
[5] Chatterjee R, Welty EV, Walder TY, Walder RY, Pruitt SL, Rogers PH, et al. Isolation and characterization of a new hemoglobin derivative cross-linked between the α chains (Lysine 99α1-99α2). J Biol Chem 1986;26:9929–37.
[6] Saxena R, Wijnhoud AD, Carton H, Hacke W, Kaste M, Przybelski RJ, et al. Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb, in acute ischemic stroke. Stroke 1999;30:993–6.
[7] Sloan EP, Koenigsberg M, Gens D, Cipolle M, Runge J, Mallory MN, et al. Diaspirin cross-linked hemoglobin (DCHLb) in the treatment of severe traumatic hemorrhagic shock. A randomized controlled efficacy trial. JAMA 1999;282:1857–64.
[8] Lamy ML, Dailey EK, Brichant JF, Larbuisson RP, Demeyere RH, Vandermeersch EA, et al. Randomized trial of diaspirin cross-linked hemoglobin solution as an alternative to blood transfusion after cardiac surgery. Anesthesiol 2000;92:646–56.
[9] Sloan EP, Koenigsberg M, Brunett PH, Bynoe RP, Morris JA, Tinkoff G, et al., DCLHb Traumatic Hemorrhagic Shock Study Group. Post-hoc mortality analysis of the efficacy trial of diaspirin cross-linked hemoglobin in the treatment of severe traumatic hemorrhagic shock. J Trauma 2002;52:887–95.
[10] Gibson JB, Maxwell RA, Schweitzer JB, Fabian TC, Proctor KG, et al. Resuscitation from severe hemorrhagic shock after traumatic brain injury using saline, shed blood, or a blood substitute. Shock 2002;17:234–44.
[11] Adamson JG, Bonaventura BJ, Song SE, Langlois SF, MacDonald ID, Moore C, et al. Production, characterization, and clinical evaluation of Hemolink, an oxidized raffinose cross-linked hemoglobin-based blood substitute. In: Rudolph AS, Rabinovici R, Feuerstein GZ, editors. Red blood cell substitutes. New York: Marcel Dekker, Inc.; 1998. p. 335–51.

[12] Carmichael FJL, Ali ACY, Campbell JA, Langlois SF, Biro GP, Willan AR, et al. A phase I study of oxidized raffinose cross-linked human hemoglobin. Crit Care Med 2000;28:2283–92.
[13] Cheng DCH. Safety and efficacy of o-raffinose cross-linked human hemoglobin (Hemolink) in cardiac surgery. Can J Anesth 2001;48:S41–8.
[14] Greenburg AG, Kim HW, 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 graft surgery. J Am Coll Surg 2004;198:373–83.
[15] http://www.hemosol.com/presspop.cfm?newsID=2063.
[16] Sprung J, Kindscher JD, Wahr JA, Levy JH, Monk TG, Moritz MW, et al. The use of bovine hemoglobin glutamer-250 (Hemopure) in surgical patients: results of a multicenter, randomized single-blinded trial. Anesth Analg 2002;94:799–808.
[17] LaMuraglia GM, O'Hara PJ, Baker WH, Naslund TC, Norris EJ, Li J, et al. The reduction of allogenic transfusion requirement in aortic surgery with hemoglobin-based solution. J Vasc Surg 2000;31:299–308.
[18] Kasper SM, Grune F, Walter M, Amr N, Erasmi H, Buzello W. 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.
[19] Standl T, Wilhelm S, Horn EP, Burmeister M, Gundlach M, Schulte am Esch J. Präoperative Hämodilution mit bovinem Hämoglobin. Akute hämodynamische Auswirkungen bei Patienten in der Leberchirurgie. Anaesthesist 1997;46:763–70.
[20] http://www.corporate-ir.net/ireye/ir_site.zhtml?ticker=bpurSA, Gould LR, Rowen AL, Sehgal HL, Moss GS. Polymerized pyridoxylated hemoglobin: a red cell substitute with normal O_2 capacity. Surgery 1984;95:433–8.
[22] Gould SA, Moore EE, Hoyt DB, Burch JM, Haenel JB, Garcia J, 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.
[23] Gould SA, Moore EE, Moore FA, Haenel JB, Burch JM, Sehgal H, et al. Clinical utility of human polymerized hemoglobin as a blood substitute following acute trauma and urgent surgery. J Trauma 1997;43:325–32.
[24] Moore EE, Gould SA, Hoyt DB, Haenel JM. Clinical utility of human polymerized hemoglobin as a blood substitute following trauma and emergent surgery. Shock 1997;7(S1):145.
[25] http://phx.corporate-ir.net/phoenix.zhtml?c=91374RM. Current status of blood substitute research: towards a new paradigm. J Int Med 2003; 253:508–17.
[27] Winslow RM. Targeted O_2 delivery by low-p50 hemoglobin: a new basis for hemoglobin-based oxygen carriers. Artif Cells Blood Substit Immobil Biotechnol 2005;33:1–12.
[28] Bjorkholm M, Fagrell B, Przybelski R, Winslow N, Young M, Winslow RM. A phase I single blind clinical trial of a new oxygen transport agent (MP4), human hemoglobin modified with maleimide-activated polyethylene glycol. Haematologica 2005;90:505–15.
[29] http://www.sangart.com/press/?pID=1045727680.
[30] Buehler PW, Alayash AI. Toxicity of hemoglobin solutions; in search of in vitro and in vivo model systems. Transfusion 2004;44:1516–30.
[31] Hess JR, Macdonald VW, Brinkley WW. Systemic and pulmonary hypertension after resuscitation with cell-free hemoglobin. J Appl Physiol 1993;74:1769–78.
[32] Keipert PE, Gonzales A, Gomez CL, MacDonald VW, Hess JR, Winslow RM, et al. Acute changes in systemic blood pressure and urine output of conscious rats following exchange transfusion with diaspirin-crosslinked hemoglobin solution. Transfusion 1993;33:701–8.
[33] Przybelski RJ, Dailey EK, Birnbaum ML. The pressor effect of hemoglobin—good or bad? In: Winslow RM, Vandegriff KD, Intaglietta M, editors. Advances in blood substitutes: industrial opportunities and medical challenges. Boston: Birkhäuser; 1997. p. 71–85.
[34] Freilich E, Freilich D, Hacker M, Leach L, Patel S, Hebert J, et al. The hemodynamic effects of diaspirin cross-linked hemoglobin in dopamine-resistant endotoxic shock in swine. Artif Cells Blood Substit Immobil Technol 2002;31:83–98.
[35] Vane LA, Funston JS, Kirscher R, Kirschner R, Harper D, Deyo DJ, et al. Comparison of transfusion with DCLHb or pRBCs for treatment of intraoperative anemia in sheep. J Apply Physiol 2002;92:343–53.
[36] Hess J, Macdonald V, Winslow R. Dehydration and shock: an animal model of hemorrhage and resuscitation of battlefield injury. Artif Cells Blood Substit Immobil Biotechnol 1992;20:499–502.
[37] Caron A, Malfatti E, Aguejouf O, Faivre-Fiorina B, Menu P. Vasoconstrictive response of rat mesenteric arterioles following infusion of cross-linked, polymerized, and conjugated hemoglobin solutions. Artif Cells Blood Substit Immobil Biotechnol 2001;29:19–30.
[38] Eich RF, Li T, Lemmon DD, Doherty DH, Curry SR, Aitken JF, et al. Mechanism of NO-induced oxidation of myoglobin and hemoglobin. Biochemistry 1996;35:6976–83.
[39] Liao JC, Hein TW, Vaughn MW, Huang KT, Kuo L. Intravascular flow decreases erythrocyte consumption of nitric oxide. Proc Natl Acad Sci USA 1999;96:8757–61.
[40] Alayash AI. Oxygen therapeutics: can we tame haemoglobin? Nat Rev Drug Discov 2004;3:152–9.
[41] Rioux F, Drapeau G, Marceau F. Recombinant human hemoglobin (rHb1.1) selectively inhibits vasorelaxation elicited by nitric oxide donors in rabbit isolated aortic rings. J Cardiovasc Pharmacol 1995;25:587–94.
[42] Ritchie AJ, Hartshorn S, Corsbie AE, Callingham BA, Latimer RD, Vuylsteke A. The action of diaspirin cross-linked haemoglobin blood substitutes on human arterial bypass conduits. Eur J Cardiothroac Surg 2000;18:241–5.
[43] Gould SA, Moss GS. Clinical development of human polymerized hemoglobin as a blood substitute. World J Surg 1996;20:1200–7.
[44] Abassi Z, Kotob S, Pieruzzi F, Abouassali M, Keiser HR, Fratantoni JC, et al. Effects of polymerization on the hypertensive action of diaspirin cross-linked hemoglobin in rats. J Lab Clin Med 1997;129:603–10.
[45] Doyle M, Apostol I, Kerwin B. Glutaralderhyde modification of recombinant human hemoglobin alters its hemodynamic properties. J Biol Chem 1999;274:2583–91.
[46] Rohlfs RJ, Bruner E, Chiu A, Gonzales A, Gonzales ML, Magde D, et al. Arterial blood pressure responses to cell-free hemoglobin solutions and the reaction with nitric oxide. J Biol Chem 1998;273:12128–34.
[47] Fitzpatrick CM, Savage SA, Kerby JD, Clouse WD, Kashyap VS. Resuscitation with a blood substitute causes vasoconstriction without nitric oxide scavenging in a model of arterial hemorrhage. J Am Coll Surg 2004;199:693–701.
[48] Winslow RM, Gonzales A, Gonzales M, Magde M, McCarthy M, Rohlfs RJ, et al. Vascular resistance and the efficacy of red cell substitutes in a rat hemorrhage model. J Appl Physiol 1998;85:993–1003.
[49] Karmakar N, Dhar P. Effect of steady shear stress on fluid filtration through the rabbit arterial wall in the presence of macromolecules. Clin Exp Pharmacol Physiol 1996;23:299–304.
[50] De Wit C, Schafer C, Von Bismarck P, Bolz SS, Pohl U. Elevation of plasma viscosity induces cell sustained NO-mediated dilation in the hamster cremaster microcirculation in vivo. Pflugers – Arch Eur J Physiol 1997;434:354–61.
[51] Intaglietta M, Johnson PC, Winslow RM. Microvascular and tissue oxygen distribution. Cardiovasc Res 1996;32:632–43.
[52] Tsai A, Friesenecker B, Winslow RM, Intaglietta M. Functional capillary density changes during blood substitution with αα-Hb and dextran 70: influence on oxygen delivery. Art Cells Blood Subs Immob Biotech 1994;22:841–7.
[53] Intaglietta M. Whitaker lecture 1996. Microcirculation, biomedical engineering and artificial blood. Ann Biomed Eng 1997;25:593–603.
[54] Rochon G, Caron A, Toussaint-Hacquard M, Alayash AI, Gentils M, Labrude P, et al. Infusion of stroma free hemoglobin at physiologically maintained viscosity delays the onset of vasoconstriction in acute normovolemic hemodilution. Hypertension 2004;43:1110–5.
[55] Rebel A, Ulatowski JA, Kwansa H, Bucci E, Koehler RC. Cerebrovascular response to decreased hematocrit: effect of cell-free hemoglobin, plasma viscosity, and CO_2. Am J Physiol Heart Circ Physiol 2003;285: H1600–H1608.

[56] Cabrales P, Tsai AG, Intaglietta M. Hyperosmotic–hyperoncotic versus hyperosmotic–hyperviscous: small volume resuscitation in hemorrhagic shock. Shock 2004;22:431–7.
[57] Cabrales P, Intaglietta M, Tsai AG. Increase plasma viscosity sustains microcirculation after resuscitation from hemorrhagic shock and continuous bleeding. Shock 2005;23:549–55.
[58] Vandegriff KD, McCarthy M, Rohlfs RJ, Winslow RM. Colloid osmotic properties of modified hemoglobins: chemically cross-linked versus polyethylene glycol surface conjugated. Biophys Chem 1997;69:23–30.
[59] Fischer S, Burnet M, Traber D, Prough DS, Kramer GC. Plasma volume expansion with solutions of hemoglobin, albumin and Ringers lactate in sheep. Am J Physiol 1999;45:H2194–H2203.
[60] Migita R, Gonzales A, Gonzales ML, Vandegriff KD, Winslow RM. Blood volume and cardiac index in rats after exchange transfusion with hemoglobin-based oxygen carriers. J Appl Physiol 1997;82:1995–2002.
[61] Guyton A. Textbook of medical physiology. Philadelphia, PA: W.B. Saunders; 1961.
[62] Duling BR, Berne RM. Longitudinal gradients in perivascular oxygen tension. A possible mechanism for the participation of oxygen in local regulation of blood flow. Circ Res 1970;27:669–78.
[63] Kerger H, TorresFilho IP, Rivas M, Winslow R, Intaglietta M. Systemic and subcutaneous microvascular oxygen tension in conscious Syrian golden hamsters. Am J Physiol 1994;268(Heart Circ. Physiol. 37): H802–H810.
[64] Tsai AG, Vandergriff KD, Intaglietta M, Winslow RM. Targeted O_2 delivery by low-P50 hemoglobin: a new basis for O_2 therapeutics. Am J Physiol Heart Circ Physiol 2003;285:H1411–H1419.
[65] Wettstein R, Tsai AG, Winslow RM, Intaglietta M. 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.
[66] Cabrales P, Tsai AG, Winslow RM, Intaglietta M. Effects of extreme hemodilution with hemoglobin-based O_2 carriers on microvascular pressure. Am J Physiol Heart Circ Physiol 2005;288:H2146–H2153.
[67] Scholander P. Oxygen transport through hemoglobin solutions. Science 1960;131:585–90.
[68] Boland E, Nair P, Lemon D, Olson J, Hellums J. An in vitro capillary system for studies on microcirculatory O_2 transport. J Appl Physiol 1987;62:791–7.
[69] Page TC, Light WR, McKay CB, Hellums JD. Oxygen transport by erythrocyte/hemoglobin solution mixtures in an in vitro capillary as a model of hemoglobin-based oxygen carrier performance. Microvasc Res 1998; 55:54–64.
[70] 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.
[71] Vaslef SN, Kaminski BJ, Talarico TL. Oxygen transport dynamics of acellular hemoglobin solutions in an isovolemic hemodilution model in swine. J Trauma 2002;51:1153–60.
[72] Sakai H, Hara H, Yuasa M, Tsai AG, Takeoka S, Tsuchida E, 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–H915.