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What happened to blood substitutes?
Qu’est il arrivé aux substituts du sang ?

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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 O₂; 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,
Oxyglobin (Biopure) (Bovine) (polymerized) Approved Veterinary-anemia, acute blood loss
PEG hemoglobin (Enzon) (Bovine) (PEG conjugated) Phase Ib radiosensitizer solid tumors
Hemolink (Hemosol) (Human) (polymerized) (discontinued) Phase II Cardiopulmonary bypass, ANH, orthopedic surgery
PolyHeme (Northfield) (Human) (PEG conjugated) Phase II Trauma, surgery
Hemospan (Sangart) (Human) (polymerized) Phase III Trauma, surgery

ANH: acute normovolemic hemodilution; ESRD: end-stage renal disease; PTCA: percutaneous transluminal coronary angioplasty.

* 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 O₂ saturation was lower suggesting that O₂ 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 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 P50, 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 P50, 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 in 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 O2 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 sub-endothelial 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 Poly-Heme 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 \(\mu\) = 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 through 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 PO₂ by matching flow to the perceived need. Paradoxically, excessive delivery of O₂ 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 O₂ content of the blood (which in turn depends on hemoglobin concentration and its degree of O₂ saturation), the ability of hemoglobin to off-load O₂ (determined in part by the P₅₀ and Hill coefficient) and the ability of O₂ 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 PO₂ as blood flows along the arterial tree and into the capillary bed is well recognized [62]. However, the loss of O₂ is particularly marked at the level of the vasoactive terminal arterioles, where the PO₂ 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 O₂ 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 O₂ to the arterial wall. The presence of hemoglobin in solution is known to enhance the diffusion of O₂ 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 O₂, as well as facilitating diffusion of O₂ 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 O₂ 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 O₂ to the arterial wall more readily. Accordingly, HBOCs with smaller molecular radii, and presumably higher diffusion coefficients for O₂, 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 O₂ delivery to the arterial wall is the oxygen affinity of the HBOC. It might be expected that an HBOC with high oxygen affinity (low P₅₀) would unload less O₂ 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 P₅₀ than a similar preparation with a low P₅₀ [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 P₅₀. Hence, the P₅₀ 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 O₂ 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.

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