Diaspirin Cross-Linked Hemoglobin and Blood Substitutes

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11.1 General Principles

Oxygen delivery (DO_2) to organs and tissues depends on flow generated by the heart (cardiac output, CO) and arterial oxygen content. Arterial oxygen content depends on oxygen partial pressure (PaO_2) and hemoglobin (Hb) concentration and saturation. In case of hypoxemia and/or low CO states, Hb concentration may play a key role in preventing tissue hypoxia and cellular dysfunction.

Although Hb concentration in perioperative settings and in critical care is a crucial aspect for almost all patients, the optimal values are still a matter of debate [1]. Nonetheless, current guidelines and recommendations suggest lower "transfusion triggers" than in the past, encouraging blood-saving techniques following a multidisciplinary, multi-procedural approach [2]. The difficulties of supplying red blood cells (RBCs), the need to overcome problems of storage and transfusion (refrigeration and crossmatching), the aim to avoid potential transfusions' harming effects (infection, transfusion reactions, transfusion-related acute lung injury, immunomodulation) [3, 4], and the need for alternatives to biological blood for religious reasons (e.g., Jehovah's Witnesses) [5, 6] have led scientists and companies, over the past three decades, to synthesize and test artificial blood solutions. Oxygen carrier (OC) is a generic definition for blood substitutes, blood surrogates, artificial Hb, or artificial blood. These substances mimic oxygen-carrying function of the RBCs (Table 11.1) and are characterized by a long shelf life. In other words, OCs are pharmacological substances that aim to improve DO_2 independently from RBCs.

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Table 11.1 The ideal oxygen carrier

Always available without temperature limitations
Long shelf life
Effective oxygen-carrying capacity
Effective volume expander
Absent scavenging effect on nitric oxide
No side effects
No infectious carrier
No crossmatching necessity
Cost-effective
Usable for cardioplegia priming and preservative fluid for transplant organs

However, OCs only transport oxygen and do not share with whole blood all its other functions (e.g., coagulation and immunological functions). Over the years, various different solutions divided into two main categories have been created and studied: hemoglobin-based oxygen carriers (HBOC) and perfluorocarbon-based oxygen carriers (PFBOC) (Table 11.2).

Both kinds of transporters bind and transport O_2, but their characteristics are totally different. During the decade 2000–2010, great enthusiasm came from the possibility to replace blood transfusions in many clinical situations and led to a number of experimental applications of these new molecules. Some of these products reached phase III in clinical trials, but unfortunately their path toward a final approval was hampered by reports on side effects and regulatory concerns about safety. As a consequence, the lacking of regulatory approval and investor supports led to the withdrawal of many products from the market.

11.2 Main Evidences

The first attempts of substituting Hb as an extracellular substance date back over 100 years ago [11–13]. Considerable side effects, with the so-called stroma-free Hb, were mainly related to renal impairment due to vasoconstriction and led to abandon these potential blood substitutes.

Hemoglobin-like oxygen carriers can be of allogeneic (from outdated red blood cells), xenogeneic (bovine), or recombinant (*E. coli*) origin [14]. Unmodified Hb solutions cannot be used because of the inherent instability of the tetrameric structure ($\alpha 2\beta 2$), which dissociates to $\alpha\beta$-dimers [15]. To stabilize the product and prevent extravasation and renal filtration, after extraction from red blood cells (stroma-free Hb), Hb molecules are modified by cross-linkage, polymerization, pyridoxylation, pegylation, or conjugation to prolong retention time and provide colloidal osmotic pressure [16, 17]. Cross-linking and polymerization appeared to have largely solved some of the problems associated with unmodified stroma-free Hb: longer half-life, limited nephrotoxicity, and improved oxygen transport [16–18].

Table 11.2 Oxygen carriers [7–10]

HBOC product	Company	Availability
Hemopure® Glutaraldehyde-polymerized bovine Hb	OPK Biotech	South Africa and Russia Expanded Access Study of HBOC-201 (*Hemopure®*) for the Treatment of Life-Threatening Anemia is currently recruiting patients *Hemopure* has not been approved yet by the FDA pending safety review
PolyHeme® Pyridoxal-50-phosphate cross-linked and glutaraldehyde-polymerized human Hb	Northfield Laboratories, Inc.	On May 9, 2009, after being informed by the FDA, the product's risks outweighed the benefits; the company shut down any research operation
HemAssist® Bis-3,5-dibromosalicyl fumarate cross-linked human Hb	Baxter Healthcare Corporation	Product withdrawn
rHb 1.1 Optro®; r Hb 2.0 Recombinant hemoglobin	Baxter Healthcare Corporation	Product withdrawn
Hemolink® Open-chain raffinose cross-linked and polymerized human Hb	Hemosol, Inc.	Abandoned due to the cardiac toxicity observed during the clinical trials
PFBOC product	*Company*	*Availability*
Oxygent® PFBOC	Alliance Pharmaceutical Corp.	European phase III in noncardiac surgery concluded in 2002 Not currently approved by the US FDA for safety reasons

Abbreviations: *HBOC* hemoglobin-based oxygen carriers, *PFBOC* perfluorocarbon-based oxygen carriers, *FDA* Food and Drug Administration, *US* United States

Although HBOCs have been shown to be effective in enhancing cellular oxygenation and improve outcome in trauma in preclinical studies [19, 20], they are no longer considered for clinical use since experimental and clinical trials have failed to prove any benefit, while severe concerns about safety have been raised. Among the HBOCs, only one, *Hemopure®* (or HBOC-201 – 13 g/dL glutaraldehyde-polymerized bovine hemoglobin), is currently available for clinical use in South Africa and Russia (Table 11.2).

11.2.1 Diaspirin Cross-Linked Hemoglobin

Sloan et al., over 15 years ago, tested the diaspirin cross-linked hemoglobin (DCLHb), a purified and chemically modified human Hb solution (*HemAssist®*, 10 g/dL diaspirin cross-linked human hemoglobin in balanced electrolytes solution) [21]. Their randomized multicenter study had the primary objective of reducing 28-day mortality for hemorrhagic shock trauma patients. The study design included

Table 11.3 Reported side effects with HBOCs in experimental and human studies [17, 23–26]

Vasoactivity-hypertension (systemic and pulmonary)	NO scavenging
Gastrointestinal	Pancreatic injury, hepatocellular injury, esophageal spasm,↑ AST, ↑ CPK, ↑ amylase, ↑ bilirubin
Renal	Heme-mediated oxidative events
Hemostasis	Coagulation defects, thrombosis, thrombocytopenia
Cardiac	Myocardial infarction

Abbreviations: *NO* nitric oxide, *AST* aspartate aminotransferase, *CPK* creatine phosphokinase

the addition of 500–1,000 mL DCLHb to standard treatment during initial fluid resuscitation. In the 58 treated patients, death rate was higher than in the 53 controls (46 % vs. 17 %; $p=0.003$). It is likely that DCLHb might have worsened outcomes by scavenging nitric oxide (NO) with worsening of hemorrhage and reduction of tissue perfusion due to vasoconstriction. Nitric oxide, an endothelial-derived relaxing factor, is a strong heme ligand, and its reduction results in systemic and pulmonary vasoconstriction, decrease in blood flow, release of proinflammatory mediators, and loss of platelet inactivation, predisposing conditions for vascular thrombosis and hemorrhage [17, 22] (Table 11.3). Nitric oxide scavenging causing microvascular vasoconstriction and reduction in functional capillary density is the major side effect for many of the HBOCs (Table 11.3). Endothelin-1, a strong vasoconstrictor produced by endothelial cells, has also been suggested to be involved in vasoconstrictor effects of HBOCs [27] together with sensitization of α-receptors [28].

In 2003, a randomized controlled study was performed by Kerner et al. [29] in trauma patients with hypovolemic shock. The study population was sorted into the standard care group ($n=62$) or into the *HemAssist*® group (1,000 mL) ($n=53$) during transport from the scene of trauma to the hospital and until definitive control of bleeding source. The trial was interrupted prematurely for futility after an interim evaluation. In fact, no difference in either 5- or 28-day organ failure or mortality between the two groups was found.

11.2.2 Other Hemoglobin-Based Oxygen Carriers

PolyHeme® (hemoglobin glutamer-256 [human]; polymerized hemoglobin, pyridoxylated; Table 11.2) was produced starting from human purified Hb, then pyridoxylated (to decrease the O_2 affinity), and polymerized with glutaraldehyde. In 1998, Gould et al. [30] first compared, in a prospective randomized trial, the therapeutic benefit of *PolyHeme*® with that of allogeneic RBCs in the treatment of acute blood loss in 44 trauma patients. *PolyHeme*® was designed to avoid the vasoconstriction issues observed with tetrameric Hb preparations, probably due to endothelial extravasation of the molecules and binding of NO. The patients were randomized to receive either RBCs ($n=23$) or up to 6 U (300 g) of *PolyHeme*® ($n=21$) as their initial blood replacement after trauma and during emergent operations. The first

results were encouraging since no serious or unexpected adverse events were related to *PolyHeme*®, which maintained total Hb concentration, despite the marked fall in RBCs Hb concentration. This led to reduction in the use of allogeneic blood [30]. In 2002, the same group of authors performed a study in massively bleeding trauma and urgent surgery [31]. A total of 171 patients received a rapid infusion of 1–20 units (1,000 g, 10 L) of *PolyHeme*® instead of RBCs as initial oxygen-carrying replacement, simulating the unavailability of RBCs. Forty patients had a nadir RBC [Hb] ≤3 g/dL. However, total [Hb] was adequately maintained because of plasma [Hb] added by *PolyHeme*®. The 30-day mortality (25 %) was compared with a similar historical group (64.5 %; $p<0.05$). On the basis of these results, the authors concluded that *PolyHeme*® should be useful in the early treatment of urgent blood loss and resolve the dilemma of unavailability of red cells. These first encouraging results led to a multicenter phase III trial performed in 2009 in the United States [32]. The study was designed to assess survival of patients resuscitated with *PolyHeme*® starting at the scene of injury. The patients were randomized to receive either up to 6 U of *PolyHeme*® during the first 12 h post-injury before receiving blood or crystalloids. After 714 patients were enrolled and randomized, 30-day mortality was higher in the *PolyHeme*® arm than in the crystalloid arm (13.4 % vs. 9.6 %), although this difference was not statistically significant. The incidence of multiple organ failure was similar (7.4 % vs. 5.5 % in *PolyHeme*® and controls, respectively). Total adverse events instead were higher in intervention vs. control group (93 % vs. 88 %; $p=0.04$); this was similar to serious adverse event, including myocardial infarction (MI) (40 % vs. 35 %; $p=0.12$).

Hemospan® (Table 11.2) is an oxygenated, polyethylene glycol-modified hemoglobin: it showed some promising results in clinical trials [15, 23]. Olofsson et al. conducted a safety phase II study in patients undergoing major orthopedic surgery. The authors compared Ringer's lactate with *Hemospan*® given before the induction of anesthesia in doses ranging from 200 to 1,000 mL. *Hemospan*® mildly elevated hepatic enzymes and lipase and was associated with less hypotension and more bradycardic events. Nausea was more common in the patients receiving *Hemospan*®, without correlation with the dose [23]. A "Phase III Study of *Hemospan*® to Prevent Hypotension in Hip Arthroplasty" has been completed, but the results have never been published [33]. Moreover, due to the lack of investor interest, this product is not currently used in clinic [34].

In the mid-1990s, recombinant technology for hemoglobin production (use of *E. coli* transfected with human hemoglobin genes; *rHb1.1*, *Optro*®) gave some promising results [35]. Nevertheless, when tested in animal models, vasoconstriction due to NO scavenging and increase in amylase and lipase levels led to project abandonment [35]. Further modification of *rHb 1.1* (*rHb 2.0*), which aimed at mitigating the vascular response [24], did not reach the desired objective, and consequently, due to the hemodynamic side effects, synthesis of recombinant product was discontinued [36].

Hemopure® (bovine hemoglobin, polymerized by glutaraldehyde-lysine) is the only available HBOC, and it is nowadays licensed in South Africa and Russia: it was tested in some clinical trials including cardiac, vascular, and surgical patients [37–39]. The largest study was a randomized controlled multicenter phase III trial

performed in 2008 in the United States. 688 patients were randomized to receive either *Hemopure*® ($n=350$) or RBCs ($n=338$) at first transfusion decision in orthopedic surgery [40]. The investigators reported that 59.4 % of the patients receiving *Hemopure*® were able to avoid allogeneic RBC transfusions; adverse events (8.47 % vs. 5.88 %; $p<0.001$) and serious adverse events (0.35 % vs. 0.25 %; $p<0.01$) were higher in *Hemopure*® in comparison with controls; mortality was comparable in the two treatment groups [40].

Hemolink® is an open-chain raffinose cross-linked and polymerized human Hb that was used in patients undergoing cardiac surgery (Table 11.2). Treatment with *Hemolink*® allowed a reduction in RBCs compared with pentastarch [41, 42]. However, hypertension, MI, increase in pancreatic enzymes, and raised bilirubin were observed [25, 41, 42]. Consequently, *Hemolink*® has been abandoned due to the toxicity observed during the clinical trials.

In 2008, Natanson et al. published a meta-analysis [17] counting 16 randomized controlled trials (3,711 patients) focusing on the safety evaluation of 5 OCs (*HemAssist*®, *Hemopure*®, *PolyHeme*®, *Hemospan*®, *Hemolink*®) in surgical, stroke, and trauma patients. Overall analysis showed a significant increase in risk of death in treated patients (relative risk (RR), 1.30; 95 % confidence interval [CI], 1.05–1.61) and risk of MI (RR, 2.71; 95 % [CI], 1.67–4.40). Although some limitations can be acknowledged (some details on study protocols were unavailable, and control groups received different treatments), this meta-analysis addressed important safety concerns as far as all five different types of OCs are concerned.

11.2.3 Perfluorocarbon-Based Oxygen Carriers

Perfluorocarbon-based oxygen carriers are inert organofluorine compounds containing only carbon and fluorine. They are chemically and biologically inert, have low viscosity, and have a high gas-dissolving capacity. Plasma half-life is approximately 12 h, and when refrigerated at 4 °C for storage, they last up to 2 years [43]. Differently from HBOCs, in PFBOC, the relationship between PaO_2 and PFC-transported O_2 is linear. Therefore, they are efficient solvents, and their oxygen-carrying capacity is relevant in patients receiving high concentrations of supplemental oxygen [43, 44]. The only product based on perfluorocarbon ever approved by the Food and Drug Administration (FDA) was *Fluosol*® in 1989, for perfusion during percutaneous coronary angioplasty [45]. In 1994 the product has been withdrawn from the market due to its insufficient applicability in clinical practice. During the following years, *Oxygent*®, a new PFBOC (Table 11.2), was tested by Spahn et al. [46] in a European phase III trial in noncardiac surgery patients, with expected blood loss of 20 mL/kg or greater, and used in conjunction with acute normovolemic hemodilution (1.8 g/Kg). The administration of *Oxygent*® as fluid for PFBOC normovolemic hemodilution reduced transfusion needs. Adverse event rates were similar in the PFBOC (86 %) and the control (81 %) groups, and the overall mortality was not statistically significant. However, more serious adverse events were reported in the PFBOC group than in the control (32 % vs. 21 %; $p<0.05$).

Conclusions

Clinical evidence, recommendations, and guidelines suggest that RBC transfusion indications should be much more restrictive than in the past and the decision to transfuse or not transfuse must be tailored on an individual basis for each patient. Nonetheless, there is an undisputed need for an oxygen-carrying product with reduced risk of transfusion harming effects, universal compatibility, infinite availability, and long-term storage capability. Perioperative settings, trauma scenes, military battlefield casualties, disaster scenarios, remote settings, and religious issues are conditions suitable for alternatives to blood administration. During the last decades, much research has been done to develop products to substitute blood transfusion: so far, randomized controlled trials have raised questions about safety and have failed to demonstrate clinical benefits of the available substitutes. Thus, new and safer alternative products are absolutely needed before transfusion medicine can be profoundly modified.

Clinical summary

Drug	Indications	Side effects	Dose	Notes
Hemoglobin-based oxygen carriers	Prehospital treatment of severe trauma Major orthopedic surgery Cardiac surgery	Vasoconstriction Pancreatic and hepatic injury Nausea Kidney oxidative injury Coagulopathy Myocardial infarction	*Hemopure®*, 1 unit = 30 g, tested to a maximum dose of 300 g	Hemoglobin-based oxygen carriers appear to increase mortality and morbidity. None of these drugs are available in Europe or the United States *Hemopure®* is available in South Africa and Russia

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