Chapter 45
Hemoglobin-Based Oxygen Carrier (HBOC) Development in Trauma: Previous Regulatory Challenges, Lessons Learned, and a Path Forward

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Abstract Historically, hemoglobin-based oxygen carriers (HBOCs) were being developed as "blood substitutes," despite their transient circulatory half-life (~ 24 h) vs. transfused red blood cells (RBCs). More recently, HBOC commercial development focused on "oxygen therapeutic" indications to provide a temporary oxygenation bridge until medical or surgical interventions (including RBC transfusion, if required) can be initiated. This included the early trauma trials with *HemAssist*® (Baxter), *Hemopure*® (Biopure) and *PolyHeme*® (Northfield) for resuscitating hypotensive shock. These trials all failed due to safety concerns (e.g., cardiac events, mortality) and certain protocol design limitations. In 2008 the Food and Drug Administration (FDA) put all HBOC trials in the US on clinical hold due to the unfavorable benefit:risk profile demonstrated by various HBOCs in different clinical studies in a meta-analysis published by Natanson et al. (2008). During standard resuscitation in trauma, organ dysfunction and failure can occur due to ischemia in critical tissues, which can be detected by the degree of lactic acidosis. Sangart's Phase 2 trauma program with MP4OX therefore added lactate >5 mmol/L as an inclusion criterion to enroll patients who had lost sufficient blood to cause a tissue oxygen debt. This was key to the successful conduct of their Phase 2 program (ex-US, from 2009 to 2012) to evaluate MP4OX as an adjunct to standard fluid resuscitation and transfusion of RBCs. In 2013, Sangart shared their Phase 2b results with the FDA, and succeeded in getting the FDA to agree that a planned Phase 2c higher dose comparison study of MP4OX in trauma could include clinical sites in the US. Unfortunately, Sangart failed to secure new funding and was forced to terminate development and operations in Dec 2013, even though a regulatory path forward with FDA approval to proceed in trauma had been achieved.

Keywords HBOC • MP4OX • Hemoglobin solutions • Trauma • Hemorrhagic shock

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1 Introduction

Hemoglobin-based oxygen carriers (HBOCs) have historically been referred to as "blood substitutes," which is a misnomer considering their transient circulatory half-life (i.e., typically ~24 h) versus transfused red blood cells (RBCs) that remain in circulation for weeks. As a result, there have been significant regulatory challenges to develop an HBOC as an alternative to RBC transfusion in clinical settings where blood transfusion is typically part of the standard of care. The risk of viral disease transmission from donor RBCs is always a concern to patients, even though the blood banking industry has implemented numerous strategies and new tests to reduce these risks to exceedingly low levels [1]. To market an HBOC for reduction or avoidance of RBC transfusion, regulators demanded compelling clinical data to demonstrate equivalent safety versus allogeneic donor RBCs. This would have required unrealistically large numbers of patients, and an unreasonable cost and time commitment. Commercial development therefore re-focused on alternative clinical scenarios where the physicochemical properties of the HBOC are used to enhance oxygen delivery to hypoxic tissues in ischemic organs. By acting as an "oxygen therapeutic" agent, the HBOC can be used as a "bridge" to transfusion, or as an alternative to RBCs in situations where transfusion is not an established therapy or may be contraindicated [2]. This provides a greater opportunity to prevent or treat ischemia-related morbidity and to potentially reduce mortality, and thereby demonstrate sufficiently compelling efficacy to satisfy the regulatory requirement for clinical benefit to gain marketing approval of an HBOC.

2 Medical Need in Trauma and Rationale for HBOCs

Mortality following blunt or penetrating injuries in trauma patients is typically caused by acute hemorrhagic shock resulting from severe and uncontrolled blood loss, and hemorrhage accounts for 30–40% of all deaths after trauma [3]. Shock is characterized by inadequate perfusion (ischemia) that leads to a shortage of cellular substrates, and insufficient oxygenation of vital organs (tissue hypoxia). Most tissues initially maintain adequate energy generation to support cellular metabolism by anaerobic glycolysis, resulting in lactic acid production in proportion to the overall oxygen debt. It is hypothesized that following severe shock, damage to the microcirculation delays restoration of normal perfusion, which is a significant contributor to worse outcome due to delayed resolution of the lactic acidosis.

Studies have shown that the severity of lactic acidosis in trauma is closely associated with worse outcomes [4, 5]. Moreover, the time needed to resolve lactic acidosis may be more important than the peak value reached, and prolonged elevation of lactate levels has been correlated with increased risk of organ failure and higher mortality [6]. Currently there is no approved medication or therapy to specifically address the altered state of the microcirculation during hemorrhagic shock. In order

for an HBOC to perform as an "ischemic rescue" therapeutic agent, it must augment oxygen transport in the microcirculation, improve homogeneity of oxygen flux in capillaries, and facilitate diffusion of oxygen from RBCs to the endothelial cells lining vessels in hypoxic tissues.

The average mortality rate in modern trauma centers has reached low levels (~ 12–15%), which makes a pure mortality-based primary outcome for an HBOC trial a difficult hurdle to achieve. For those trauma patients in severe hemorrhagic shock who also exhibit higher levels of blood lactate (> 5 mmol/L) due to critical tissue ischemia, a recent analysis by Lefering et al. (2013) showed that mortality rates are about two-fold higher (~ 25–30%) [7]. This higher-risk patient population therefore represents an opportunity for HBOC treatment, given as an adjunct to standard-of-care resuscitation, to improve survival as part of a composite clinical efficacy endpoint to demonstrate patient benefit in late-stage clinical trials.

3 Clinical Experience: Lessons Learned with HBOCs in Trauma

Several different HBOC formulations that demonstrated efficacy in preclinical animal models of hemorrhagic shock resuscitation, have also been tested in clinical trials in trauma: Baxter's α-α diaspirin crosslinked human Hb (*HemAssist*®), Biopure's glutaraldehyde-polymerized bovine Hb (*Hemopure*®), Northfield's glutaraldehyde-polymerized human Hb (*PolyHeme*®), and Sangart's maleimide-pegylated human Hb (MP4OX). The important lesson that has been learned in the past 10–15 years from these studies is that the physicochemical properties of each HBOC (e.g., lower P50, higher COP, degree of pegylation, type of chemical modification, molecular size, lower Hb concentration, physiological viscosity) play a role in determining both its safety profile, and whether a particular HBOC will enhance microvascular perfusion in critical organs and restore oxygenation of ischemic tissues sufficiently to reverse any accumulated oxygen debt. Despite success in preclinical animal models, several early-generation HBOC formulations (Baxter, Biopure, and Northfield) with higher P50 and lower COP were unable to translate their preclinical efficacy into patient benefit in late-stage human trauma trials [8].

One of the frequently observed adverse events (AEs) associated with earlier generation HBOCs was hypertension, caused by vasoconstriction secondary to scavenging of nitric oxide. This vasoconstriction effect had the unfortunate consequence of compromising the intended benefit of administering an HBOC to treat tissue ischemia in trauma. Perhaps more importantly, trial design issues played a role in limiting the ability of earlier HBOC trauma studies to demonstrate compelling clinical efficacy. By defining inclusion criteria that were overly broad, these studies allowed for inclusion of patients at both ends of the injury spectrum, i.e., those severely injured and likely to die no matter what intervention was given, and those with non

life-threatening injuries who survive regardless of treatment. Using the degree of hypotension based on a patient's systolic blood pressure (SBP) < 90 mmHg as a criterion for inclusion may have limited proper evaluation of the severity of hemorrhagic shock, resulting in poor patient selection, potential under-resuscitation, and possibly inappropriate dosing. The NORTHFIELD study also withheld potentially life-saving RBC transfusions for 12 h post-injury. These protocol design issues represent shortcomings of the BAXTER and NORTHFIELD trauma studies that may be partly to blame for their inability to demonstrate sufficient efficacy. When combined with the observations that the incidence of some serious adverse event (SAE) rates (including MI, hypertension, coagulopathy) and mortality were higher in HBOC-treated patients, it became impossible to achieve a favorable demonstration of clinical benefit and produced negative opinions and rejections from regulatory authorities. After their Phase 3 programs failed, both BAXTER and NORTHFIELD decided to terminate their HBOC development due to insufficient funding to pursue new studies and based on other commercial and business considerations.

SANGART's recent Phase 2 trauma program with MP4OX added an important design feature missing from previous protocols, i.e., an elevated blood lactate ≥5 mmol/L as a physiological biomarker for inclusion at randomization to prospectively select patients who had suffered sufficient blood loss to reach a critical level of tissue ischemia [9]. SANGART completed a 51-patient pilot Phase 2a feasibility study in 2010 to compare two doses, and a 329-patient multi-center, randomized, single-dose Phase 2b study in 2012. Both of these studies were double blinded, and demonstrated the safety and potential efficacy of a low dose of MP4OX given as an adjunct to standard fluid resuscitation and blood product therapy (i.e., RBCs, FFP, platelets, as needed) in severely injured trauma patients. The Phase 2a study demonstrated more rapid reversal of lactic acidosis and a correlation with better outcomes when lactate levels normalized (≤ 2.2 mmol/L) within 8 h or when lactate decreased by ≥20% in 2 h [10]. In the Phase 2b study, a numerically higher percentage of patients treated with MP4OX were alive and discharged from hospital at Day 28 (primary efficacy endpoint) versus controls (57% vs. 50%; $p = 0.18$). Overall mortality in the MP4OX-treated patients was slightly lower compared to controls (11.6% vs. 13.9%; $p = 0.73$), which represents the first and only trauma study to demonstrate fewer deaths in HBOC-treated patients. There were no differences in the frequency of SAEs or AEs between the two groups. Multiple secondary endpoints also showed promising trends in the MP4OX group (i.e., fewer days on ventilator, in the ICU and in the hospital, as well as faster times to complete resolution of organ failure) [11].

In hindsight, the Phase 2b study was underpowered to confirm the efficacy of the 250-mL MP4OX treatment. One shortcoming of the SANGART Phase 2 program was the premature selection of a single low dose for the Phase 2b study, based on insufficient dose escalation to evaluate higher doses in the Phase 2a feasibility study, which resulted in the need for an additional follow-up study to complete a proper dose comparison. As a result, a 570-patient double-blind, controlled, Phase 2c dose-comparison protocol was designed to determine whether treatment with a 500-mL or 750-mL dose of MP4OX versus standard-of-care might improve patient outcomes and demonstrate compelling efficacy in severely injured trauma patients with evidence of lactic acidosis due to hemorrhagic shock.

4 Regulatory Challenges, Safety, and a Pathway Forward

Previous regulatory guidance from the Food and Drug Administration (FDA) had suggested sponsors demonstrate safety of HBOCs in clinically stable elective surgery patients before moving to high-risk trauma patients [12]. Ironically, it is precisely in trauma patients where an HBOC with appropriate properties may have the greatest opportunity to show clinical benefit, as these patients present with a significant burden of morbidity and risk of death from severe hemorrhagic shock and the adverse ischemic consequences of tissue hypoperfusion. In April 2008, a conference sponsored by the FDA, the Dept. of Defense, and the National Institutes of Health (NIH) was convened to review the current status and safety of various HBOCs in development. The FDA's position and premise for holding this conference at that time was that similar SAE profiles amongst several HBOC products were raising questions regarding the possibility of common underlying mechanism(s) of toxicity despite differences between these HBOC formulations. A variety of opinions were expressed regarding these safety concerns; however, most experts suggested that the biggest challenge for the field was to identify appropriate clinical situations where there would be a more favorable balance of benefit to risk for HBOCs, and finding appropriate methods to evaluate the efficacy and safety of HBOCs in these settings [13].

Coincident with the FDA workshop, was the release of a publication by Natanson *et al*. reporting a meta-analysis to evaluate the relative risk of MI and death in patients enrolled in various HBOC trials [14]. Despite significant methodological issues and questionable statistical validity of a statistical analysis that aggregated disparate trials from a variety of patient populations (i.e., elective surgery vs. emergency or trauma), with different controls (patients receiving crystalloids vs. colloids vs. blood products), and HBOC preparations with diverse physicochemical properties, Natanson concluded that HBOCs were associated with an increased relative risk of death and MI. Due to concerns raised by this meta-analysis, the FDA imposed an immediate clinical hold on all HBOC trials that were either ongoing or planned in the USA at that time. Not surprisingly, several scientific experts and commercial HBOC developers challenged the validity of the methodology and conclusion(s) of this meta-analysis and the FDA's reaction to it [15].

Fortunately, regulatory authorities within the European Union (EU) and in multiple countries worldwide disagreed with the FDA-imposed moratorium, and decided to allow clinical studies to continue after performing their own internal safety review of all relevant clinical and preclinical data provided to them. At that time, Sangart decided to re-focus their clinical development from elective orthopedic surgery to a high-risk trauma indication where MP4OX would have a greater opportunity to demonstrate patient benefit, using Sangart's clinical data from two previous Phase 3 trials in hip arthroplasty [16, 17] as a supporting safety database. Regulators in South Africa, France, Germany, and the UK approved the Phase 2a pilot trauma study in 2009. Subsequently, from 2011 to 2012, Sangart also completed a larger Phase 2b trauma trial after successfully obtaining regulatory approvals in 14 countries worldwide (not including the USA).

While the FDA was intrigued by the success of the Phase 2a trial and the safety profile for MP4OX in that study, they were still unwilling to allow US sites to participate in the Phase 2b trauma trial. However, the FDA was eager for Sangart to show them the results from the Phase 2b study. To help the FDA understand that their pegylated and high affinity MP4OX formulation was different from most early-generation HBOCs, Sangart agreed with the FDA's request for a new submission to summarize all relevant biophysical characterization data, preclinical hemodynamic pharmacology and oxygenation findings, and any new clinical results available from the trauma program. A follow-up pre-IND submission for a new Phase 2c trauma study to compare two higher doses of MP4OX was submitted to the FDA in 2013, and the FDA agreed that Sangart could include US sites in this international trial.

Unfortunately, this positive opinion from the FDA came too late for Sangart to initiate patient enrollment in the Phase 2c study, because a failure to secure new funding forced Sangart to terminate development and cease operations in December 2013. Nevertheless, efforts are still underway to secure new funding to re-establish clinical development of MP4OX and other HBOC formulations for potential future applications in trauma and other ischemia-related clinical settings.

5 Future Directions and Indications for HBOC Development

There have been many potential clinical indications proposed for using HBOCs to prevent or treat acute ischemic conditions [18]. These cover a range of potential applications: [i] protection/maintenance of the functional integrity of vital organs at risk from various medical conditions and/or surgical procedures, e.g., brain (stroke, TBI), spinal chord (vascular surgery), heart (MI, cardiac arrest, angioplasty, CPR, bypass surgery), kidney (transplant surgery), and gut (surgery, shock); [ii] oncology applications to enhance oxygenation of solid tumors (during radiation and/or chemotherapy); [iii] organ transplantation (*ex vivo* perfusion to prolong storage hold-time for heart, lung, kidney, or liver), [iv] drug delivery (targeting oncology drugs conjugated to Hb to the liver); [v] ischemic limbs (peripheral vascular disease, diabetes); [vi] wound healing; [vii] Sickle Cell Disease (acute vaso-occlusive crisis); [viii] sepsis (refractory hypotensive shock); [ix] acute hemolytic anemia (oxygenation bridge); [x] veterinary use (due to limited availability of species-specific animal blood); and [xi] compassionate use (as a temporary blood substitute) for life-threatening anemia when RBCs are not available.

A number of ischemia-related indications are being pursued by Prolong Pharmaceuticals (South Plains, NJ) in early-stage clinical trials using their pegylated bovine carboxyHb (*Sanguinate*™). By correcting oxygenation levels and down-regulating inflammation, *Sanguinate* may have the potential to effectively treat many of the debilitating comorbidities of Sickle Cell disease (SCD) and other disorders caused by anemia and/or hypoxia/ischemia [19]. These include preventing delayed graft function following kidney transplantation, treating painful vaso-

occlusive crises in adult patients with SCD and beta-Thalassemia, and reducing or preventing delayed cerebral ischemia following subarachnoid hemorrhage.

Recent research in the field has focused on the oxidative properties of Hb and how they can be modified to reduce the potential intrinsic toxicity of exogenously added iron and heme when HBOCs are infused. One such approach, developed by SynZyme Technologies (Sioux Falls, SD) [20] involves polynitroxylation of a pegylated Hb to create PNPH (aka *SanFlow*, or nanoRBC) as a nanomedicine for use in critical care and resuscitation following hemorrhagic shock [21]. Polynitroxylation adds superoxide dismutation activities to the Hb, which when infused create a superoxide-free vascular space. This helps to conserve endogenous nitric oxide levels within the vasculature, thereby preventing vasoconstriction and maintaining microcirculatory blood flow. PNPH has been evaluated in preclinical animal studies in stroke [22] and traumatic brain injury (TBI) [23], and has demonstrated both safety and neuroprotective properties (i.e., prevent neuronal death, restore MAP, reduce brain edema and increase cerebral perfusion pressure) following resuscitation in a murine model of combined TBI plus hemorrhagic shock [24].

Traumatic hemorrhage from penetrating and/or blunt injury offers a huge potential market for an HBOC as an adjunct to early resuscitation in both military and civilian settings. Similarly, non-traumatic hemorrhagic shock represents a potential expansion of the trauma indication, by using HBOC treatment following cerebral bleeding, ruptured aortic aneurysms, iatrogenic hemorrhage during vascular surgery, or obstetric bleeding from a ruptured placenta. TBI represents a subset of trauma with perhaps the highest mortality and longest hospital and ICU stays. Any HBOC that can treat oxidative stress from superoxide, down-regulate inflammation, and deliver oxygen to ischemic tissue holds great promise as a potential therapeutic agent in trauma, TBI, SCD, and possibly in stroke.

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