HemAssist: Development, Clinical Trials, Lessons Learned

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Introduction

HemAssist® development was initiated under the auspices of a contract between the United States Army Medical Research and Development Command (USAMRDC) and Baxter International Corporation from 1985 to 1988 [1]. This effort entailed a close collaboration between researchers at the Letterman Army Institute of Research (LAIR) and Baxter. During the course of this effort a mutual decision was made to focus on the production of a specific HBOC discovered at the University of Iowa [2]. The active principle was human hemoglobin crosslinked between the two alpha subunits by a fumarate crosslinker derived from the diaspirin reagent, bis (3,5 dibromosalicyl) fumarate (DBBF). Hence, this product was initially denoted as diaspirin crosslinked hemoglobin (DCLHb). This particular derivative was chosen because a high yield was obtained of a specific hemoglobin derivative which was stabilized to enhance intravascular persistence and exhibited oxygen binding and release characteristics which closely mimicked those of fresh whole blood [2, 3].

Production scale up of DCLHb entailed the identification and implementation of purification procedures which could be performed at large scale, development of an improved method for the synthesis of large quantities of highly purified DBBF, development and validation of both in-process and final release assays, and the integration and validation of virus removal and inactivation steps [4]. The resulting process consistently produced DCLHb from human red cells with an overall yield in excess of 50%, and which was sterile, highly crosslinked, and exhibited low levels of non-hemoglobin proteins and process residuals [4]. As a consequence of these efforts, over 200 liters of 10 g/dl DCLHb solution was delivered to LAIR [1]. This material was used in a variety of preclinical assessments at LAIR [1, 5, 6] and laboratories receiving material from LAIR [7–9]. Ultimately, commercial scale production of DCLHb was demonstrated in a purpose built facility in Neuchatel, Switzerland, and validation studies demonstrated a total level of virus reduction of at least 100 billion fold for an array of virus challenges [10, 11].

In addition to the experiments performed at LAIR, a number of studies were performed at Baxter, as well as academic and contract laboratories collaborating with Baxter. Results were sufficiently encouraging that Baxter senior management decided to invest considerable company resources into the further development of DCLHb after the conclusion of the contract with USAMRDC. The US ARMY also decided to pursue further assessment of DCLHb using material produced at LAIR and, subsequently, the Walter Reed Army Institute of Research [12, 13]. The production of DCLHb by the ARMY was initially based on the complete set of standard operating procedures which was conveyed to the ARMY as part of the Baxter/USAMRDC contract. However, both the ARMY and Baxter made subsequent changes to their respective manufacturing processes so that these diverged significantly [4, 12, 13]. On the basis of published data, the Baxter product was more highly crosslinked and contained lower levels of contaminating pyrogens and phospholipids (Table 27.1). The ARMY also changed the composition of their final formulation, replacing lactate with acetate [12, 13]. Acetate has pharmacologic activities which have led to discontinuation of its use in dialysis due to myocardial depression, hypotension, hyopnea, and hypoxemia [14].

Table 27.1 Comparison of DCLHb (HemAssist) produced by Baxter and ααHb produced by the US Army

Parameter (Units)	Army[a]	Baxter[b]
Total hemoglobin (g/dL)	9.8–9.95	10.2
Methemoglobin (%)	3.2–7.5	3.2
Desired product (%)[c]	50–90+	99.8
Endotoxin (EU/mL)	0.1–0.2	<0.06
Rabbit pyrogen test (% pass)	63–100	100
Phospholipid (ppm)	0.75–1.0	0.1

[a]Data taken from references [12, 13]. [b]Data taken from reference [4]. [c]Defined as the amount of desired alpha-alpha crosslinked hemoglobin

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Thus, it is not surprising that these two, ostensibly similar, HBOC formulations exhibit different physiologic properties, the most striking of which is the fact that the Baxter product causes significant blood volume expansion after infusion [15], whereas the ARMY product causes blood volume contraction [16, 17]. Unfortunately, the distribution of two different diaspirin crosslinked formulations amongst researchers has caused confusion in the field, especially since these have sometimes been identified by the same nomenclature (e.g. αα crosslinked hemoglobin). All subsequent observations discussed in this chapter are based on results obtained with the Baxter manufactured product which is denoted interchangeably as DCLHb or HemAssist.

Preclinical Testing

The availability of large quantities of high quality product enabled extensive safety and efficacy testing. Efficacy was demonstrated in animal models of high volume blood replacement [18], resuscitation from hemorrhagic shock [19], resuscitation from cardiac arrest [20], treatment of cerebral [21] and spinal cord [22] ischemia, and support of cardiac function during balloon angioplasty [23]. DCLHb was also well tolerated during extensive whole animal and organ specific toxicity testing. Transient increases in some enzymes were observed, but these resolved rapidly. Centrolobular necrosis was detected in the liver, but this also resolved rapidly. Notably, DCLHb had little adverse effect on kidney pathology or function [3]. However, two other issues were identified during the preclinical testing of DCLHb which prompted further investigations.

The first of these was the fact that DCLHb was vasoactive, resulting in elevations of blood pressure in excess of those explicable on the basis of blood volume replacement or expansion [24]. Subsequent characterization of this phenomenon demonstrated that consumption of nitric oxide (NO) by extravasated hemoglobin is the primary mechanism for this hypertension [25]. Physiologic measurements using radioactive microspheres showed that vasoconstriction after DCLHb infusion varies significantly between organs and tissues, being pronounced in skeletal muscle, less in many other tissues, and, importantly, nonexistent in heart vessels [26]. The pressor response to DCLHb is manifested at low infused concentrations and rises to a maximum of approximately 40% of baseline at intermediate doses, with no further increase at higher DCLHb concentrations [24]. This increase could be mitigated by commonly used antihypertensive agents. Ultimately, the regulatory position on this issue was that patient risk was sufficiently low that clinical trials could proceed, albeit with close monitoring of hemodynamics.

A second finding of concern was the appearance of microscopic cardiac lesions in certain species [27]. This finding prompted an extensive testing program to characterize pathology, explore potential mechanisms, and define consequences. These studies ruled out coronary vasoconstriction and infarction as mechanisms, and demonstrated that the lesion resulted in no detectable adverse effect on cardiac electrophysiology or function, even in the most sensitive species. In light of these results the risk of significant harm to humans was deemed acceptable and clinical testing was initiated. During this time period DCLHb was trade named "HemAssist™".

Clinical Testing

Low doses of HemAssist infused into normal volunteers were well tolerated, with modest, but expected, increases in blood pressure and no evidence of adverse cardiac events [28]. On the basis of these results, as well as those of preclinical evaluations, it was decided to focus subsequent clinical trials on the indications of blood replacement and resuscitation from hemorrhagic shock.

HemAssist was evaluated as a replacement for blood transfusion in both cardiac [29] and noncardiac surgery patients [30, 31]. In cardiac surgery patients, the overall number of packed erythrocyte units (pRBCs) transfused did not differ between treated and control groups, but 19% of treated patients did avoid any pRBC infusion (versus 0% of controls) when up to 750 mL of HemAssist was utilized [29]. Treated patients experienced modest increases in mean arterial and pulmonary artery pressures and a decrease in cardiac output, but these were not associated with clinical sequelae. Jaundice/hyperbilirubinemia, hematuria, and increased liver enzymes were expected observations in treated patients as a consequence of increased hemoglobin metabolism. There was no elevation in cardiac damage specific enzymes relative to controls, but some increase in lipase and pancreatic-specific amylase. No cases of pancreatitis were observed in this study.

In a small trial with noncardiac surgery patients, there was no evidence of cardiac ischemia, cardiac infarction, stroke, or pulmonary edema, although blood pressure was elevated for 24–30 hours after DCLHb infusion [30]. More (7/12) DCLHb treated patients were treated with antihypertensives than controls (2/12). Jaundice, elevated bilirubin levels, and asymptomatic hematuria were again observed more frequently in treated patients, as were transient elevations in serum lactate dehydrogenase, aspartate transaminase, creatine kinase, and amylase. Renal function appeared to be well maintained, but three treated patients experienced ileus and one was diagnosed with mild pancreatitis.

The most rigorous assessment of DCLHb in the noncardiac surgical setting was a randomized, prospective, double-blinded Phase III trial by Schubert and coworkers [31].

Utilization of up to 750 mL of DCLHb solution in lieu of blood transfusion resulted in complete avoidance of the latter in 23% of treated patients after seven days. A statistically significant ($p = 0.002$) reduction in overall pRBC transfusion was also observed, with an average of two units required in treated patients versus three in controls. Out of the 181 patients enrolled at 19 clinical sites, mortality (4% versus 3%) and the incidence of adverse events (21% versus 15%) were similar between DCLHb treated patients and controls, respectively. However, the incidence of jaundice, urinary side effects, and pancreatitis was more frequent in patients infused with HemAssist. This study was terminated early due to safety concerns about pancreatitis and a mortality imbalance observed during initial patient enrollment in a contemporaneous study of DCLHb in trauma victims.

With respect to the latter, HemAssist was evaluated in three clinical trials in the treatment of hemorrhagic shock. In a Phase II safety study, patients with Class II-IV hemorrhagic in hypovolemic shock within were infused with 50, 100 or 200 mL of a 10% DCLHb solution or normal saline, followed by standard of care [32]. Lower mortality was observed in the DCLHb versus control patients (13/71 versus 16/68), but the difference was not statistically significant. The incidence of adverse events and serious adverse events was similar between groups. There was no evidence of renal insufficiency or cardiac ischemia in treated patients. At the 200 mL dose there were greater increases in serum amylase, lactate dehydrogenase, and creatine kinase myocardial subfraction levels in treated patients, but these were not considered to be clinically significant. On the basis of these results, two Phase III studies were initiated in the US and Europe [33, 34]. Although the same formulation was used in both studies, there were differences in the clinical protocols and clinical outcomes. The US study was halted after the infusion of only 98 patients total, out of a planned 850, due to the fact that 24 treated patients had died, compared to 8 in the control arm of the study. This outcome ultimately resulted in the decision to terminate all DCLHb development. However, a subsequent analysis of patient deaths in the US trial published 3 years later by the lead study investigators suggested that all but two of the 32 patient deaths would be expected on the basis of the type and severity of patient injuries, one in each group [35]. Interestingly, a substantial fraction of the mortality imbalance occurred in the initial patients treated at the 16 study sites that eventually enrolled at least one patient [36]. Also notable is the fact that mortality in the European trial after a comparable level of enrollment (121 patients) was not significantly different between treatment and control groups (42% and 38%, respectively) [34]. Nevertheless, the European trial was terminated as a consequence of the US trial experience. Further analysis of the US trial data suggested that vasoactivity was not an important contributor to the mortality imbalance as the average blood pressure immediately after resuscitation was quite similar between treated and control patients [37]. Moreover, DCLHb infusion did not result in higher lactate or base deficit levels, implying no adverse effects on organ perfusion [38].

Although the small number of patients enrolled in the Phase III resuscitation trials does not permit definitive conclusions, comparison of differences between the US and European protocols (Table 27.2) suggest several interesting hypotheses [39]. One difference is that the US trial protocol permitted the enrollment of patients who had suffered cardiac arrest, while such patients were excluded from the European study. Experience has shown that such patients have a greater than 90% chance of mortality [40]. As it happened, 12 such patients were enrolled in the US study, but the majority of these (10 versus 2) were randomized to the treatment arm [35]. Another potentially important difference was that treatment of patients was begun on-scene in the European study, but only after admission to the emergency room in the US. A third difference was that European patients were excluded from enrollment if they had received one liter or more of other fluids, while no such restriction was placed on US patients. As a consequence, US patients may have received substantially more total fluid volume than their European counterparts [39]. This is particularly noteworthy in light of subsequent results obtained in sheep resuscitation protocols which showed that HemAssist was a much more potent volume expander than a comparable volume of oncotically matched albumin solution [41]. Thus, the combination of an unexpectedly large HemAssist volume expansion effect coupled with larger infused volumes of additional fluids could have resulted in over resuscitation of some treated patients in the US trial. Yet another potentially important difference is the fact that US patients suffered more penetrating injuries. As noted by Kerner et al., such patients may be particularly susceptible to aggressive fluid resuscitation [34]. Finally, it is notable that the mortality in both treated groups and the European control group were similar to the targeted patient mortality risk of 40%, but the mortality in the US control group was significantly

Table 27.2 Comparison of protocols and outcomes in Phase III trials of HemAssist (DCLHb) in the US and Europe[a]

Study	Europe	US
Site of treatment	On-scene	Trauma center
Volume DCLHb infused (mL)	250–1000	500–1000
Limitation on previous fluid volume Infused	Yes	No
Previous cardiac arrest as exclusion Criteria	Yes	No
28-day mortality:		
Treated (deaths/n)(%)	22/52 (42%)	24/52 (46%)
Controls (deaths/n)(5)	22/58 (38%)	8/46 (17%)

[a]Data taken from references [33, 34]

lower, suggesting some confounding factors were present in the US trial [33]. A more comprehensive discussion of HemAssist clinical development has been presented by Przybelski [42].

Subsequent Analyses – Myocardial Infarction

Although HemAssist development was terminated in 1999, data from clinical trials were included in a meta-analysis of HBOC toxicity [43]. This analysis suggested that HBOCs as a class increase the risk of mortality and myocardial infarction (MI) in treated patients. With respect to HemAssist, the mortality imbalance derives almost entirely from the US Phase III trauma study, which, as noted above, is significantly confounded. Thus, the mortality risk associated with the use of this product is unclear.

More suggestive conclusions may be drawn with respect to myocardial infarction, assuming that the reported incidence of MI in the HBOC clinical literature is positively correlated with the actual occurrence of MI [44]. This qualifier is prompted by the fact that hemoglobin and hemoglobin metabolites may interfere with the analysis of troponin, a primary marker of MI in contemporary cardiology. With this reservation, additional comprehensive analysis of clinical trial results implies that HBOCs as a class probably do increase the risk of MI in certain patients because this risk is positively correlated with both HBOC dose and molecular weight [44]. Consideration of possible mechanisms suggests that vasoconstriction is unlikely, since HBOCs do not reduce coronary blood flow [44]. MI risk is also probably unrelated to the generation of microscopic heart lesions, because these lesions were never found to be associated with areas of infarct [27]. Furthermore, the molecular weight dependency of MI risk between different HBOCs is exactly opposite that for microscopic lesion development [27, 44]. However, other mechanisms are possible, including the enhancement of intravascular thrombosis risk due to exacerbation of endothelial dysfunction by heme released from oxidized hemoglobin, platelet activation due to the consumption of nitric oxide and/or oxidative stress, and inhibition of the breakdown of ultralarge von Willebrand factor [45]. Intravascular etiology is supported by the positive correlation between HBOC molecular weight and MI incidence, since larger HBOCs are known to persist in the circulation longer [46], and therefore any untoward interactions within the vascular space or with endothelium are more likely to occur. Thus, HemAssist, like other chemically modified HBOCs, is probably associated with increased risk of MI, a risk which was not apparent during earlier analyses of individual clinical trial data.

Summary and Lessons Learned

Highly purified solutions of DCLHb (HemAssist) were efficiently produced at large scale and performed well in standard safety testing in healthy animals. In addition, efficacy was demonstrated in a variety of animal models of stroke and hemorrhagic shock. The safety profile also seemed acceptable in Phase I and II human trials, but several issues were identified in more demanding Phase III studies. This suggests that a more systematic progression into the latter is appropriate, because the learning curve is steep [36], particularly with a totally new class of products. HBOCs are different from both red cells and traditional intravenous solutions and it is unclear whether the protocols used for HemAssist were optimal, despite substantial efforts expended in their design and implementation [34, 35, 41]. Several clinicians have argued that higher doses may have been appropriate in the resuscitation of some patients [34]. Thus, future HBOC clinical trials should be designed with regard to posology, patient selection and concomitant therapies (e.g. intravenous fluids) in light of these results.

With respect to safety, it is remarkable that at doses which far exceed those of other pharmacological agents, HemAssist was generally well tolerated in a variety of animal models and human patients. Nevertheless, HemAssist was not devoid of risk, and it should not be surprising that a new finding would emerge as a larger body of patients was evaluated. The incidence of MI in control patients enrolled in HemAssist clinical trials averaged 1%, while in treated patients it was 1.6%. With other HBOCs the incidence in treated patients was higher [44]. Thus, there is legitimate cause for clinical and regulatory concern with respect to the safety of these products. On the other hand, the fact that most treated patients do not experience MI, there is demonstrable blood sparing in some patient populations [31], and anecdotal evidence that HBOCs can support cardiac function and sustain life in dire situations [47, 48], suggests that this class of therapy merits further development. Aside from the continued development of HBOC derivatives with improved safety characteristics, better understanding of how to best use these products and more refined selection of those patients most likely to benefit from HBOC treatment are warranted.

Key Points

- HemAssist development demonstrated that a highly crosslinked, highly purified human HBOC formulation

with extremely low risk of pathogen transmission could be efficiently produced at large scale.

- Different formulations of diaspirin crosslinked hemoglobin produced in different facilities may elicit different physiologic responses.
- Preclinical testing demonstrated low toxicity when HemAssist was infused into healthy animals from a variety of species and the ability to reverse whole animal and specific organ ischemia in models of hemorrhagic shock, stroke, and organ perfusion.
- In human testing, HemAssist was well tolerated at low doses in normal human volunteers and moderate doses in most patients.
- Blood sparing was demonstrated in some, but not all, patient populations.
- The increase in systemic blood pressure as a consequence of the vasoactivity of HemAssist was generally well tolerated and manageable.
- Several safety concerns arose during later stage clinical testing suggesting that HemAssist infusion may be associated with an increased risk of pancreatitis, myocardial infarction, and mortality in some patients.
- Comparison of the protocols and patient enrollment criteria between US and European Phase III trials of HemAssist in the treatment of hemorrhagic shock suggest that higher treated patient mortality in the former was due to an imbalance with respect to risk between treated and control patients and, possibly, suboptimal fluid management.
- There is a substantial learning curve associated with the optimal use of HBOCs in extremely ill patients.

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