

# **Hemoglobin Based Oxygen Carriers in Trauma**

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The ideal intravenous fluid for trauma resuscitation would have the following properties: provide volume expansion, carry oxygen, possess a long shelf life at room temperature, not affect coagulation, and be universally compatible, non-antigenic, non-infectious, and inexpensive. No fluid meets all of these requirements, however hemoglobin based oxygen carriers (HBOCs) currently in the advanced stages of development, bring us closer to the ideal. FDA approval for several of these carriers may occur within the next two to three years. Despite the theoretical promise and attraction that these compounds possess, their true utility remains largely unexplored.

HBOCs are a member of a relatively new group of compounds known as oxygen therapeutics. These are oxygen-carrying compounds which can be used to augment or replace the oxygen carrying capacity of red blood cells (RBCs). Perfluorocarbon emulsions are the other class of compounds within this group and they function by carrying dissolved oxygen rather than binding it. Currently, in order to transport significant quantities of oxygen, perfluorocarbons require high-inspired oxygen concentrations, thus requiring intubation. In contrast, HBOCs carry significant quantities of oxygen at ambient oxygen partial pressures. For this reason, HBOCs, at least for now, have greater potential in the trauma setting, especially for pre-hospital use.

HBOCs have been tested in a variety of settings to temporarily augment oxygen carrying capacity without utilizing red blood cell transfusion, including trauma resuscitation, intraoperative autologous blood donation in cardiac surgery, and resuscitation during surgery involving large blood loss. When used during active hemorrhage, HBOCs can temporarily maintain adequate oxygen carrying capacity and intravascular volume until hemorrhage is controlled, at which point red blood cells can be used as necessary. Used in this way, HBOCs have the potential for reducing exposure to banked blood and its associated negative immunomodulatory effects and infectious risks as well as maximizing the use of a finite resource.

HBOC's are particularly attractive for trauma resuscitation. Often, large amounts of banked blood are necessary during initial resuscitation to maintain adequate oxygen delivery until hemorrhage is controlled. In trauma patients, transfusion of more than 6 units of packed RBCs during initial resuscitation efforts has been shown to be an independent predictor of multi-organ failure<sup>1</sup>. This effect is thought to be due to pro-inflammatory effects of substances present in banked blood. Therefore, reducing exposure to banked blood during trauma resuscitation should improve outcome. In theory, HBOCs have this capability.

Hemoglobin for use in HBOCs is obtained either from out-dated human packed RBCs or from bovine RBCs by lysis and filtration. The filtration process is particularly important as even a small amount of residual RBC stroma (cell membrane and other non-

hemoglobin RBC components) is nephrotoxic secondary to thrombosis of the renal vasculature. Hemoglobin obtained in this manner is referred to as stroma free hemoglobin (SFH) and is the precursor of current HBOCs.

The hemoglobin in HBOCs acts very much like the hemoglobin contained in RBCs, both carry oxygen by binding it. Patients treated with HBOCs during ongoing hemorrhage will lose RBC hemoglobin which is replaced by HBOC hemoglobin. This process can continue to very low levels of RBC hemoglobin, less than 1 g/dl. Clearly the well-known relationship between hematocrit and hemoglobin is no longer applicable during HBOC therapy, thus making hemoglobin and not hematocrit the parameter of choice for assessment of oxygen carrying capacity.

The oxygen-carrying component common to all HBOCs is a chemically altered polymerized acellular hemoglobin. Hemoglobin is a 64 kD tetrameric protein composed of two alpha and two beta subunits, each containing an iron based heme group which is capable of binding one molecule of oxygen. One gram of hemoglobin is capable of binding up to 1.36 ml O<sub>2</sub>. The binding and release of oxygen are complex and are described by the oxygen hemoglobin dissociation curve. The P<sub>50</sub>, the partial pressure of oxygen that results in the hemoglobin being 50% saturated, is an important descriptor of hemoglobin oxygen affinity. The P<sub>50</sub> of normal hemoglobin within red blood cells is 27 mm Hg. In contrast, hemoglobin in solution (removed from RBCs) has a much higher affinity, P<sub>50</sub> of 12 mm Hg, due mainly to the lack of 2,3 diphosphoglycerate<sup>2</sup>.

The concentration of hemoglobin in a unit of packed RBCs is approximately 20 g/dl in a volume of approximately 250 ml. Thus the total hemoglobin in one unit of packed RBCs is approximately 50g. HBOC are packaged so that one unit of HBOC contains between 25 - 50 g of hemoglobin in volumes of 250 to 500 ml. The HBOC is suspended in an isotonic crystalloid solution.

## **Development of HBOCs**

The two major efforts in the development of HBOCs have been eliminating the major adverse effects caused by SFH infusion and optimizing the physical properties of the hemoglobin solution to carry and release oxygen at clinically relevant levels.

The first reported attempt at using acellular hemoglobin solutions as a replacement for RBCs in dogs dates back to 1934 when it was shown that dogs and cats exchanged transfused with SFH to a hematocrit (HCT) of zero maintained their oxygen consumption<sup>1</sup>. Interest in the subject revived during the 1970's and it was during this period that more was learned about the major adverse effects of SFH administration which include vasoconstriction and nephrotoxicity.

Soluble hemoglobin's adverse hemodynamic effects result from the fact that hemoglobin binds nitric oxide with high affinity. Nitric oxide, produced by vascular endothelial cells, is a major mediator of vascular smooth muscle tone and diffuses from the endothelial cell into the vessel lumen and into the vessel wall. The nitric oxide that diffuses into the blood

within the lumen is normally bound by hemoglobin and therefore normally plays no role in vasomotor regulation. However, tetrameric hemoglobin in solution can diffuse from the blood into the vessel wall where it binds nitric oxide within the interstitium surrounding the vascular smooth muscle cells resulting in vasoconstriction and concomitant significant increases in mean arterial pressure. Renal vascular vasoconstriction may also play a role in the negative renal effects.

Hemoglobin tetramers in SFH are unstable in solution and can dissociate into monomers and dimers. In the kidney, these hemoglobin monomers and dimers filter across the glomerulus and are toxic to the renal tubules resulting in transient decreased renal function. The kidney is particularly sensitive; human volunteers given low doses of SFH, acellular hemoglobin concentration of only 57 mg/dl, experienced a 50% decrease in creatinine clearance<sup>3</sup>. This information clearly precludes the use of unmodified SFH as an oxygen carrier since clinically relevant doses of hemoglobin (7-10g/dl) are over one hundred times the concentration achieved in that study.

To overcome both of these negative effects, polymerization was used to create hemoglobin macromolecules (poly-SFH), which, due to their increased size, do not diffuse across the glomerulus or the vascular endothelium. In the case of nephrotoxicity, this has been entirely successful. In contrast, vasoconstriction is still a clinical issue for some of the HBOCs being tested currently and is thought to be due to residual unreacted tetramer remaining in the product.

### Optimizing the physical properties of SFH

Unmodified SFH has a  $P_{50}$  of 12 mm Hg and a colloid osmotic pressure (COP) of 70 mm Hg at a hemoglobin concentration of 15 g/dl. Initially, efforts to decrease the oxygen affinity of SFH allowing it to release oxygen to tissues at acceptable  $PO_2$  levels involved the addition of 2,3 diphosphoglycerate (2,3 DPG) to the solution. This was unsuccessful since the 2,3 DPG was rapidly degraded in the blood following administration<sup>1</sup>. Subsequent efforts involve chemical modification of the hemoglobin at or near the 2,3 DPG binding site to raise the  $P_{50}$ . These efforts have been successful and the  $P_{50}$ 's of HBOCs currently under investigation are between 30-32 mm Hg, higher than that of RBC hemoglobin!

In addition to reducing these negative effects mentioned above, polymerization dramatically reduces the COP of SFH solutions while having no effect on oxygen carrying capacity. For example, a SFH solution containing 15 g/dl of hemoglobin has a COP of 70 mm Hg. Following polymerization to form poly-SFH, the COP is reduced to 25 mm Hg while still retaining 15g/dl of hemoglobin. The degree of polymerization however is limited by increases in solution viscosity. Currently available HBOCs have a hemoglobin concentration of 10 – 13 g/dl and viscosities of between 1 and 2 centipoise (cp). For comparison, the viscosity of water is 1.0 cp, plasma is 1.6 cp, hetastarch solutions are 2 cp, and blood at HCT of 45% is 4 cp.

## HBOCs in Late Stages of Development

Currently, in the United States, there are three HBOCs in phase III trials in a variety of clinical settings: Poly-SFH-P (PolyHeme, Northfield Laboratories), HBOC-201 (Hemopure, Biopure Inc.) and o-raffinose cross-linked hemoglobin (Hemolink, Hemosol Inc)<sup>13,15,16,28</sup>. Their properties are listed in table 1. Many properties are similar, but there are important differences.

An extremely low level of free tetramer, less than 1%, makes PolyHeme unique among HBOCs. The presence of free tetramer is associated with nitric oxide binding and vasoconstriction. So far, PolyHeme is the only HBOC that does not cause an increase in mean arterial pressure and this is thought to be due to the low levels of tetramer. This low tetramer level is specified in Northfield Laboratories' PolyHeme patent so it is unclear whether other HBOC manufacturers will ever be able to produce low tetramer product<sup>4</sup>.

The free tetramer/vasoconstriction issue is important and most likely played a role in the demise of Baxter Inc.'s HBOC product, diaspirin linked hemoglobin (HemAssist) in 1998. This product consisted entirely of stabilized hemoglobin tetramers and caused significant blood pressure increases in both trauma patients and cardiac patients. A phase III trial of HemAssist vs. standard resuscitation in trauma patients was interrupted due to increased mortality in the HemAssist group vs. the control group<sup>5,6,7</sup>. Following this, Baxter discontinued further development of this product.

Both Hemopure and Hemolink contain significant quantities of free hemoglobin tetramer, 5% and 35% respectively. Hemopure causes significant increases in mean arterial pressure (MAP) in surgical patients<sup>8</sup> and aortic surgery<sup>9,10</sup>. Both Hemopure and Hemolink raise MAP in cardiac surgery<sup>11,12</sup>. In addition to the increase in MAP, compared to hetastarch, Hemopure also caused significantly increased systemic vascular resistance (SVR), pulmonary vascular resistance and decreased cardiac index (CI) in patients undergoing aortic surgery. These changes resulted in a significant decrease in oxygen delivery of the patients receiving Hemopure vs. hetastarch<sup>9</sup>. This result is troubling and may bode ill for Hemopure's future.

Another difference is the source of hemoglobin (Table 1). Instead of using human hemoglobin as a precursor, Hemopure utilizes bovine hemoglobin offering several advantages. It is more readily available and the supply is seemingly inexhaustible. Bovine hemoglobin's P<sub>50</sub> (38 mm Hg) is naturally greater than human hemoglobin and therefore does not require chemical modification to the hemoglobin prior to polymerization<sup>13</sup>. Downsides to the use of a non-human source include the potential for allergic reaction/ antibody formation and disease transmission such as the prion mediated bovine spongiform encephalopathy. In studies to date, no allergic reactions have occurred and no significant antibody formation to the product as occurred. There are no reports of patients having repeated exposures to the product. Biopure Inc., the manufacturer of Hemopure, maintains a disease free herd which should reduce the risk of

cross infection<sup>13</sup>. At this stage it appears that the risks of a bovine hemoglobin source are more theoretical than real.

Shelf life and storage conditions are other important differences between the products. Shelf life of all HBOCs is limited by methemoglobin formation. Methemoglobin is formed when the iron atom of the heme group becomes oxidized to the ferric state. Heme in this condition is unable to carry oxygen. All HBOCs are prone to methemoglobin formation over time as the enzyme system within RBCs, methemoglobin reductase, is absent. Hemopure is stable for at least 3 years at room temperature while PolyHeme and Hemolink have shorter shelf lives and require refrigeration. This gives Hemopure a potential advantage in the pre-hospital trauma resuscitation (both civilian and military) arena where transport logistics play a role.

All HBOCs currently under investigation have limited intravascular persistence. Intravascular half-life of 18- 24 hours is typical. This underscores the fact that they are only useful as a temporizing therapy. HBOCs are cleared by the reticuloendothelial system. Concerns have been raised regarding overload of this system as well as overload of transferrin and haptoglobin but there is no evidence of problems up to doses of 20 units, 1000g<sup>29</sup>.

## **Problems Associated with HBOCs**

Nephrotoxicity is no longer an issue. Vasoconstriction due to nitric oxide scavenging as well as other mechanisms, while still present with some HBOCs, has been reduced compared to SFH. Time and further study will determine if even modest vasoconstriction may still pose a problem for the widespread use of some HBOCs.

There are several other issues associated with HBOC administration. Most of these issues are common to all HBOCs. Methemoglobinemia occurs following HBOC administration and the severity appears to be dose dependent. Peak methemoglobin levels occur 3 to 4 days post-infusion. Little was seen immediately after infusion indicating heme iron oxidation occurs while in the circulation. Peak plasma methemoglobin levels of 7% occurred after a 2.5 g/kg hemoglobin dose of Hemopure in surgical patients, ie 7% of plasma hemoglobin (not total hemoglobin) was oxidized<sup>8</sup>. Since any methemoglobin formed within RBCs is enzymatically reduced, the methemoglobinemia seen after HBOC infusion is due solely to oxidation of HBOC hemoglobin and not RBC hemoglobin, and thus limits only the oxygen carrying capacity of the HBOC hemoglobin. Methemoglobinemia following HBOC administration is usually by itself not severe enough to interfere with pulse oximetry. However, methemoglobinemia may contribute to the direct interference with pulse oximetry caused by high levels of HBOCs themselves.

HBOCs interfere with pulse oximetry in a dose dependent manner, unrelated to methemoglobin. There is only one published study of the effect which showed at low doses, up to 1.36g/dl plasma hemoglobin of Hemopure, there was no difference in

oxygen saturation as determined by pulse oximetry vs. blood gas analysis<sup>14</sup>. There is no published information at higher more clinically relevant doses, 5 – 8 g/dl. From clinical experience, higher levels of PolyHeme, 5-7 g/dl cause variable but dose dependent decreases in pulse oximeter readings despite high arterial PO<sub>2</sub> and high saturation by arterial blood gas analysis. Pulse oximeter readings between 78% - 85% are common in patients following administration of multiple units of PolyHeme despite adequate oxygenation shown by blood gas.

Interference from HBOC therapy can eliminate pulse oximetry as a useful monitor of oxygenation. Since pulse oximetry is a ubiquitous and often heavily relied upon monitor, its loss not only impacts clinical care but also makes one realize just how much one relies on this monitor. More frequent blood gas analysis is required in patients who have received HBOCs to assess oxygenation particularly in unstable or dynamic situations.

HBOCs have no effect upon either platelet function or function of the coagulation cascade. However, coagulation disturbances can be seen clinically earlier when using HBOCs vs. banked blood in massive transfusion situations, ie over 6 to 8 units. This is due to the fact that packed RBC units contain approximately 40 ml of plasma and therefore some coagulation factors whereas HBOC units contain no plasma, the HBOC being suspended in an isotonic crystalloid solution. After the administration of an equivalent hemoglobin dose, 10 units for example, there would be no plasma given with 10 units of HBOC while there would be approximately 400 ml of plasma (10 units x 40 ml plasma/unit) given with 10 units of packed RBCs, almost the equivalent of 2 units of fresh frozen plasma (FFP). Therefore, massive resuscitation using HBOCs will require not only earlier administration of FFP compared to that of banked blood but most likely will require larger amounts as well.

Most patients given HBOCs will develop splotchy red/purple skin discoloration, the severity of which is dose dependent. This may be due to HBOC extravasation into the skin and subcutaneous tissues. This discoloration fades over approximately a week following infusion and appears to be without any clinical significance.

Conscious patients report gastrointestinal distress with HBOC administration. The incidence is unclear but appears to be low and is thought to be due to GI dysmotility induced by nitric oxide scavenging by the HBOC hemoglobin<sup>13</sup>. This phenomenon is most likely of limited clinical significance.

HBOCs can interfere with routine clinical laboratory tests including but not limited to: serum electrolytes, creatinine, BUN, liver enzymes, bilirubin, total protein, prothrombin time (PT), partial thromboplastin time (PTT) and lactate<sup>17,18</sup>. The amount of interference is dependent upon the assay being performed, the HBOC, the concentration of HBOC, and the specific laboratory equipment in use. Analyzers utilizing optical/absorbance methods are affected to a greater degree than other types of instruments<sup>19</sup>. When HBOCs are used, laboratory personnel should be made aware so that they are able to apply the necessary corrections to ensure accurate results.

Blood gas, hematocrit and platelet determinations are unaffected. Standard hemoglobin testing returns the total hemoglobin concentration within the sample, both RBC and plasma (HBOC) hemoglobin. A plasma hemoglobin assay is required to assess HBOC hemoglobin levels. A rough estimate of HBOC hemoglobin level can be obtained if the hemoglobin and hematocrit are known by subtracting the RBC hemoglobin from the total, ie HBOC hemoglobin concentration (g/dl) = total hemoglobin (g/dl) – (HCT(%) / 3).

Free hemoglobin has neurotoxic effects in vitro<sup>13</sup>. Because of concern that HBOC hemoglobin extravasation into central nervous system structures may result in neurological damage, all studies involving HBOCs have excluded patients with significant head injury. There are no published animal studies testing HBOCs in the setting of head injury. This lack of information is unfortunate particularly when dealing with trauma. Until more information becomes available, HBOCs should not be administered to head injured patients.

HBOC therapy is accompanied by a modest, dose dependent increase in serum bilirubin concentrations which typically occur 3 to 4 days post infusion<sup>20</sup>. This appears to be clinically insignificant and is secondary to breakdown of the HBOC hemoglobin in the reticuloendothelial system.

## **HBOCs in Trauma**

### **Polyheme**

PolyHeme and HemAssist are the only HBOCs for which there are published studies involving trauma<sup>28</sup>. As mentioned above, trauma resuscitation using Hemassist increased mortality precipitating the end of its development.

Polyheme has been tested in trauma resuscitation, abdominal aortic aneurysm repair (unpublished) and sickle cell crisis (case report). A phase II study<sup>20</sup> in 39 trauma victims showed that PolyHeme (up to 6 units, 300 g hemoglobin) was able to maintain adequate hemoglobin levels despite loss of RBC hemoglobin due to hemorrhage. Indeed, the patients who received 6 units of PolyHeme had an average nadir RBC hemoglobin concentration of 2.9 g/dL. The fact that 37% of the oxygen carried by Polyheme hemoglobin to the tissues was extracted demonstrated the ability of PolyHeme to load and unload oxygen. This oxygen extraction ratio was significantly higher than that of RBC hemoglobin, 27%, with the difference most likely being due to PolyHeme's higher P<sub>50</sub>. Safety data including temperature, heart rate, mean arterial pressure, creatinine clearance, liver function tests showed no significant changes although there was an expected trend toward increased levels of bilirubin 2 to 3 days post infusion. Importantly, there was no evidence for any vasoconstrictive effects. 59% of patients avoided PRBC transfusion in the first 24 hours.

A prospective phase III study<sup>21</sup> randomized 44 trauma patients to receive either PolyHeme (up to 6 units) or PRBCs when increased oxygen carrying capacity was

indicated during initial resuscitation. There were no adverse events related to PolyHeme and no significant differences in temperature, heart rate, mean arterial pressure and creatinine between the two groups. Again, the expected rise in bilirubin occurred 2 to 3 days post infusion. While total hemoglobin remained the same for each group, the PolyHeme group had a transient and significantly lower RBC hemoglobin than the control group with peak plasma hemoglobin concentrations averaging 3.9 g/dl. The PolyHeme group received significantly less PRBCs through day 1 with a trend toward receiving less PRBCs at day 3 ( $p=.06$ ) compared to the control group.

A third study<sup>22</sup> involving 171 trauma patients was designed to simulate the situation where blood might be unavailable. This study involved using up to 20 units (1000g hemoglobin, 10 L) of PolyHeme for initial resuscitation and compared the 30-day mortality of the group to that of historical controls, a group of 300 patients who experienced surgical hemorrhage and refused blood products on religious grounds. Nadir post PolyHeme infusion RBC hemoglobin concentrations in the experimental group were compared to the data from the historical control group. PolyHeme significantly improved survival in those patients whose RBC hemoglobin levels fell below 5.3 g/dl. Historically, a hemoglobin level of 2 g/dL or less has a mortality of 100%. 42 PolyHeme patients had nadir RBC hemoglobin levels less than or equal to 2 g/dL yet 31 survived. While the comparison with a historical control group of completely different composition has its limitations, there are no other control groups available for this type of analysis.

A phase III randomized controlled multicenter study is underway to compare the outcomes of trauma patients randomized to receive up to 6 units of PolyHeme, beginning pre-hospital, vs. standard resuscitation (crystalloid and banked blood).

Two other studies involving trauma patients and PolyHeme have shown a favorable immunomodulatory effect in patients receiving PolyHeme in lieu of PRBCs. In trauma patients, transfusion of more than 6 PRBCs is an independent predictor of the development of multi-organ failure (MOF). This is thought to be due in part to priming of neutrophils by substances contained in banked blood exacerbating the hyperinflammatory state. One study showed significantly less early neutrophil activation in patients receiving PolyHeme vs. those receiving PRBCs<sup>23</sup>. The other study showed significantly lower levels of proinflammatory cytokines in PolyHeme treated patients vs. the PRBC group<sup>24</sup>. Whether this translates into improved outcome remains to be seen.

## **Hemopure**

Hemopure has been studied in animals in trauma models. In a swine liver crush model with hemorrhage of 50 ml/kg, Hemopure not surprisingly was superior to hetastarch resuscitation and no fluid resuscitation, allowing 96-hour survival in 7/8 swine vs. 0/14 in the other groups<sup>25</sup>. In a hypotensive resuscitation model following controlled hemorrhage in swine, Hemopure proved as effective as Lactated Ringers and blood<sup>26</sup>.

There are no published studies involving Hemopure in humans for trauma resuscitation. Hemopure has been approved for use as an 'oxygen bridge' in South Africa making it the

first HBOC approved for human clinical use, however, at the present time, no information is available on its use<sup>30</sup>.

### **Hemolink**

Hemolink has only been studied in animal models as far as trauma resuscitation is concerned. In a hamster hemorrhagic shock model, Hemolink was as effective as blood and superior to non-oxygen carrying solutions in restoring acid base and microcirculatory status<sup>27</sup>. Recently a phase III trial of Hemolink in cardiac surgery was stopped due to an increased incidence of adverse myocardial events in the Hemolink group thus putting Hemolink's future in question.

### **Summary**

Hemopure and Hemolink have not been studied in the setting of trauma in humans. Will they be as efficacious as PolyHeme? The answer will most likely depend on the free tetramer issue. Both contain greater amounts of tetramer, and both have been shown in other clinical settings to cause modest increases in SVR and arterial pressure. Just as with HemAssist, increased amounts of tetramer may negatively affect their clinical performance in trauma resuscitation. However, HemAssist was composed entirely of stabilized tetramers while Hemolink and Hemopure contain less than 5% tetramer. Further study of these compounds in the trauma setting is required however, both have a long way to go to catch up to PolyHeme.

Whether these HBOCs live up to their theoretical potential in trauma resuscitation and other settings remains to be determined. With several pivotal studies underway, the verdict may come within the next 1-2 years. In addition, as with all new therapies, if approved for use, economics will undoubtedly play a role in their clinical application.

Table 1  
HBOC Physical Properties (adapted from ref. 28)

HBOC	Poly-SFH-P	HBOC 201	o-raffimer cross-linked hgb
Tradename	<b>Polyheme</b>	<b>Hemopure</b>	<b>Hemolink</b>
Manufacturer	Northfield Laboratories Inc.	Biopure Inc.	Hemosol Inc
Hemoglobin Source	Human	Bovine	Human
Polymerizer	gluteraldehyde	gluteraldehyde	o-raffinose
Hemoglobin (g/dL)	10	13	10
Unit Volume (mL)	500	250	250
Hemoglobin (g) per Unit	50	30	25
P <sub>50</sub> (mmHg)	28-32	38	39+/-12 31+/-6 26 +/-4
Colloid Osmotic Pressure (mm Hg)	20-25	17	
Osmolarity(mOsm)		290-310	
Viscosity (cp)	1.9-2.2	1.3	1 –2
Tetramer % (< 64 kD)	<1	<5	<66
Methemoglobin %	<8%	<10%	<15
Shelf Life 4°C 21°C	>6 weeks >1.5 years	> 3years >2 years	>1 year -
T <sub>1/2</sub> (hrs)	24	19	18

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