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Hemoglobin-Based Oxygen Carriers in Trauma Care: Scientific Rationale for the US Multicenter Prehosptial Trial

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Abstract

Background: The greatest need for blood substitutes worldwide is in patients with unanticipated acute blood loss, and trauma is the most likely scenario. The blood substitutes reaching advanced clinical trials today are red blood cell (RBC) substitutes derived from hemoglobin. The hemoglobin-based oxygen carriers (HBOCs) tested currently in advanced clinical trials are polymerized hemoglobin solutions.

Methods: In the USA, the standard approach to restoring oxygen delivery for hemorrhagic shock has been crystalloid administration to expand intravascular volume, followed by stored RBCs for critical anemia. Allogeneic RBCs, however, may have adverse immunoinflammatory effects that increase the risk of postinjury multiple organ failure (MOF). Phase II in hospital clinical trials, as well as in vitro and in vivo work, suggest that resuscitation with an HBOC—in lieu of stored RBCs—attenuates the systemic inflammatory response invoked in the pathogenesis of MOF. Specifically, an HBOC has been shown to obviate stored RBC-provoked polymorphonuclear neutrophil (PMN) priming, endothelial activation, and systemic release of interleukins (IL) 6, 8, and 10. In a 2-event rodent study of shock-induced PMN-mediated acute respiratory distress syndrome (ARDS), the simulated prehospital administration of an HBOC markedly attenuated lung injury.

Results: Based on this background and work by others, we have initiated a US multicenter prehospital trial in which severely injured patients with major blood loss [systolic blood pressure (SBP) $\leq$ 90 mmHg] are randomized to initial field resuscitation with crystalloid versus HBOC. During the hospital phase, the control group is further resuscitated with stored RBCs whereas the study group receives HBOC (up to 6 units) in the first 12 hours. The primary study endpoint is decreased 30-day mortality, and secondary endpoints include reductions in administration of allogeneic RBCs and uncrossmatched RBCs; avoiding circulating hemoglobin levels <5 g/dl; and decreased ARDS and MOF.

Conclusions: To date, >500 injured patients have been enrolled in this multicenter trial, and the final interim analyses support the original target of 720.

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The current generation of blood substitutes tested in clinical trials are red blood cell (RBC) substitutes, *i.e.,* they are designed primarily to transport oxygen. The products now being used in advanced-phase clinical trials

Table 1.
Potential clinical benefits of hemoglobin-based oxygen carriers in trauma care

Availability
Abundant supply
Universally compatible
Prolonged shelf life
Storage at room temperature
Safety
No disease transmissions
No antigenic reactions
No immunologic effects
Efficacy
Enhanced oxygen delivery
Improved rheologic properties

are derived from hemoglobin (Hb) and thus are often referred to as hemoglobin-based oxygen carriers (HBOCs). The potential benefits of HBOCs are well known (Table 1). The objectives of this brief overview are to outline emerging applications of HBOCs in trauma care and review the scientific background for ongoing patient trials in the USA

POTENTIAL ROLE OF HEMOGLOBIN-BASED OXYGEN CARRIERS IN TRAUMA CARE (TABLE 2)

The US Food and Drug Administration (FDA) approval of a new product proceeds through phases I, II, and III studies designed to establish safety and efficacy. FDA regulation defines efficacy as follows: ''Effectiveness means a reasonable expectation that . . . the pharmacologic or other effect of the biologic product . . . will serve a clinically significant function in the diagnosis, cure, mitigation, treatment or prevention of disease in man.''[1] The Center for Biologics Evaluation and Research (CBER) is the review body for the FDA in the arena of biology and has published a comprehensive listing of ''points to consider in the safety evaluation of HBOCs.''[2] These points encompass characterization of the product, animal safety testing, and human studies and address the theoretic concerns of Hb solutions raised previously,[3–6] including pulmonary and systemic hypertension, organ dysfunction, oxidative tissue injury, synergy with bacterial pathogens, and immunomodulation. In 1994, CBER convened a workshop with the National Heart, Lung and Blood Institute and the Department of the Army to develop ''points to consider in the efficacy evaluation of HBOCs.''[7] Documenting a direct clinical endpoint for HBOCs is challenging because this endpoint has never been established for RBCs. Specific recommendations for clinical studies are in 3 areas: perioperative applications, acute hemorrhagic shock, and regional perfusion. Field trials for postinjury hemorrhagic shock, where RBCs are not available, are difficult because of safety and ethical issues. Decreased perioperative allogeneic RBC transfusion is regarded as a clinical benefit, but the potential risks of HBOCs have to be defined and evaluated as well. Regional perfusion studies include reperfusion following ischemia; for example, as an adjunct during coronary angioplasty (the FDA approved Fluosol DA in 1989 as an O_2-carring drug for this setting).

CLINICAL EVALUATION OF MODIFIED TETRAMERIC HEMOGLOBIN IN TRAUMA CARE: THE FIRST MULTICENTER TRIAL

Of the modified Hb tetrameric solutions that looked promising in the late 1980s, one formulation was authorized by the FDA for a phase III study in trauma. HemAssist (Baxter Healthcare, Boulder, CO, USA) consisted of Hb tetramers cross-linked between alpha subunits with bis(3,5 dibromosalicyl) fumarate to prevent dissociation into dimers and reduce oxygen affinity. Unfortunately, this clinical trial failed.[8] Regarded by some as a major setback for HBOCs, it is important to emphasize that this US multicenter trial of diaspirin cross-linked Hb (DCLHb) for the treatment of severe traumatic hemorrhagic shock was based on the explicit proposal that ''DCLHb was tested not as a substitute for blood but rather as an adjunct to the currently used therapies for enhancing oxygen delivery: fluids, blood, and operative intervention.'' Although the unexpected outcome raised the issue of comparable study groups, the difference in the primary study endpoint was that the 28-day mortality for the DCLHb group was 46% (24 of 52) compared with 17% (8 of 46) for the control [normal saline (NS)] group. Much expert thought and preparation went into the study design of this human trial, but the scientific justification of using a vasoconstricting agent for the initial resuscitation of acute hemorrhagic shock was questionable in retrospect.

The authors rationalized this study design because in preclinical trials ''DCLHb has been shown to be effective in enhancing perfusion in small volumes, suggesting a pharmacologic effect that is independent of hemoglobin.'' But the pharmacologic effect was not always reported as beneficial. In 1993, Hess *et al.*,[9] at the Letterman Army Institute of Research, reported that in a swine model of hemorrhagic shock, DCLHb infusion doubled systemic and pulmonary vascular resistance, and these responses were associated with a fall in cardiac output. In fact, these

Table 2.
Potential role of hemoglobin-based oxygen carriers in trauma care

Application	Location
Perioperative applications	
Reduce allogeneic RBC transfusions	ED, angiography, OR, ICU
Attenuate transfusion immunomodulation	OR, ICU
Acute hemorrhagic shock	
When stored RBCs unavailable	Field, ED, OR, ICU, remote hospital, civilian disaster, military conflict
More efficient resuscitation	Field, ED, OR, ICU
Low-volume resuscitation	Remote hospital, civilian disaster, military conflict
Regional perfusion	
Enhance O_2 delivery	
Ischemic reperfusion tissue/organ	OR, ICU
Inflamed tissue	OR, ICU
Ex vivo organ perfusion	Hospital, OR

RBC: red blood cells; ED: emergency department; OR: operating room; ICU: intensive care unit.

Table 3
Characteristics of current hemoglobin-based oxygen carriers in phase III trials

Characteristic	Hemopure	PolyHeme	RBCs
Hemoglobin (g %)	13	10	13
Unit equivalent (g)	30	50	50
Molecular weight (>64 kDa)	≥95%	≥99%	≥100%
P_{50} (mmHg)	38	29	26
Hill coefficient	1.4	1.7	2.7
Oncotic pressure (mmHg)	25	23	25
Viscosity	1.3 cp	2.1 cp	(Whole blood = 5–10 cp)
Methemoglobin (%)	<10	<8	<1
Half life	19 hours	24 hours	31 days
Shelf life @ 4°C	≥3 years	≥1.5 years	42 days
Shelf life @ 21°C	≥2 years	≥6 weeks	≥6 hours

RBC: red blood cells; Cp: centipoises; P_{50}: tension when hemoglobin-binding sites are 50% saturated.

changes were equivalent to resuscitation with unmodified tetrameric Hb. The authors concluded: ''The decrease in cardiac output associated with the vasoconstriction in the Hb-treated animals was equal to the increase in oxygen-carrying capacity – crystalloid or colloid solutions provided equally rapid correction of the elevated whole blood lactate.'' In a follow-up study,[10] the infusion of low-dose (4 ml/kg =14 g Hb) DCLHb into swine subjected to hemorrhagic shock prompted the authors to further warn that ''pulmonary hypertension and low peripheral perfusion may offset benefits for trauma patients.'' Although the authors of the DCLHb trial cited several animal models that appeared to support their study hypothesis, none of these models replicated their study design—a lesson for future conduct of clinical trials with HBOCs.

The mechanisms responsible for vasoconstriction resulting from DCLHb administration were investigated before the trauma clinical trial. The increased vascular resistance was believed to be predominantly mediated by the scavenging of nitric oxide (NO) with an additional component of enhanced endothelin release.[11–13] Subsequently, alternative mechanisms have been proposed, including release-enhanced adrenergic receptor sensitivity and reduced arterial wall shear stress secondary to decreased viscosity.[14–15] Development of DCLHb has been terminated, but the relevance of these basic mechanisms to future trauma care with HBOCs is clear.

CLINICAL SAFETY OF POLYMERIZED HEMOGLOBIN IN TRAUMA CARE: THE NEW GENERATION

At this moment, the HBOCs currently tested in Phase III trials are polymerized Hb solutions (Table 3). Polymerization addresses several of the problems inherent in tetrameric Hb. *i.e.,* short intravascular retention and reduced colloid osmotic activity. Polymerization also appears to attenuate vasoconstriction associated with the infusion of Hb solutions. A proposed explanation is that tetrameric Hb (65 kDa) extravasates through the endothelium to bind abluminal NO, leading to unopposed

vasoconstriction; but polymerized Hb (>130 kDa) remains in the vasculature to bind only luminal NO. Of interest, Hb of the common earthworm, *Lumbricus terrestrics*, is a polymer with a molecular weight of 400 kDa that circulates extracellularly.[16] Mice and rats undergoing exchange transfusion with this naturally occurring polymeric Hb showed no changes in behavior, and nuclear magnetic resonance spectroscopy of the heart confirmed normal O_2-carrying capacity.[17]

Polymerized HBOCs have undergone extensive preclinical and clinical testing for safety. Hemopure (Biopure Corp, Cambridge, MA, USA), a polymer of bovine Hb, has been used successfully to reduce allogeneic RBC transfusion in elective cardiac,[18] aortic,[19] and hepatic[20] surgery. One study with abdominal aortic reconstruction raised concern about increased systemic vascular resistance,[21] an effect identified in normal volunteers.[22] Recent animal studies designed to replicate prehospital hypotensive resuscitation for hemorrhagic shock have been encouraging.[23–25] Hemopure has been approved in South Africa for replacement of acute blood loss, but there are no published results to date. PolyHeme (Northfield Lab, Evanston, IL, USA) has been evaluated predominantly in acutely injured patients. PolyHeme is derived from outdated human stored blood. After lysis of RBCs, the native tetrameric hemoglobin is polymerized with glutaraldehyde. Pyridoxal phosphate is used to obtain a more physiologic P50. The meticulous, multistep biochemical purification of PolyHeme is believed to eliminate the risk of infection transmission. Under FDA guidance, we initiated clinical trials in trauma to confirm safety with escalating doses of PolyHeme. In the first clinical trail[26], 39 patients received 1 (n = 14), 2 (n = 2), 3 (n = 15), or 6 (n = 8) units of PolyHeme as their initial resuscitation after acute blood loss. Infusion rates ranged from 1 unit in 175 minutes to 6 units (300 g) in 20 minutes. Although the RBC Hb fell to 2.9 ± 0.2 g%, total Hb was maintained at 7.5 ± 0.2 g% with PolyHeme. With respect to safety, the patient's temperature, mean arterial pressure, heart rate, and creatinine clearance did not change during the 72-hour study period. Liver function tests and amylase varied substantially because of patient injuries. Cognizant of the vasoconstriction associated with the DCLHb clinical trial, we designed a study to specifically evaluate the vascular response to PolyHeme infusion in acutely injured patients.[27] Patients requiring urgent transfusion were administered either PolyHeme (up to 6 units) or stored RBCs during their initial resuscitation. Systemic arterial pressure, pulmonary arterial pressure, cardiac index, and pulmonary capillary wedge pressure were measured every 4 hours postinfusion. There were no significant differences between the groups for these indices or the calculated systemic or pulmonary vascular resistance. Additional issues reported with the clinical use of polymerized Hb solutions include interference of laboratory tests that are based on colorimetric changes from dissolved plasma Hb, inaccuracy of O_2 saturation monitoring because of methemoglobin, mild elevations of serum amylase (but without evidence of pancreatitis), and skin rashes. None of these have been considered clinically significant adverse events to date.

CLINICAL EFFICACY OF POLYMERIZED HEMOGLOBIN IN TRAUMA CARE

Perioperative Applications: Reduce Allogeneic RBC Transfusions in Trauma Care

Prompted by FDA guidelines to demonstrate efficacy, all HBOC companies have pursued what appeared to be the simplest clinically, *i.e.,* to reduce the need for allogeneic RBC transfusions. In collaboration with David B. Hoyt, MD and the University of California at San Diego, we conducted a randomized trial in patients requiring urgent transfusion.[28] The 44 trauma patients [Injury Severity Score (ISS) = 21 ± 1.3) were allocated to receive stored RBCs or up to 6 units of PolyHeme as their initial blood replacement. The RBC Hb was equivalent preinfusion (10.4 ± 0.4 g% vs. 9.4 ± 0.3 g%); at end infusion, the RBC Hb of the PolyHeme patients fell to 5.8 ± 0.5 g% versus 10.6 ± 0.3 g% in the control. The PolyHeme group received 4.4 ± 0.3 units, resulting in a plasma Hb of 3.9 ± 0.2 g%. The total number of allogeneic RBC transfusions for the control versus PolyHeme was 10.4 ± 0.9 units versus 6.8 ± 0.9 units ($P < 0.05$), respectively, through day 1, and 11.3 ± 0.9 units versus 7.8 ± 0.9 units ($P = 0.06$), respectively, through day 3. After the initial phase, infusion of 4.6 units of stored RBCs in the control group was equivalent to the 5.2 units in the PolyHeme group. Both volumes presumably represented the infused RBCs or PolyHeme lost during acute hemorrhage before operative control. Subsequent replacement volumes were comparable, ultimately sparing the PolyHeme group approximately 4 units of allogeneic RBC transfusion.

Perioperative Applications: Reduce Allogenic RBC Transfusions During Initial Resuscitation and Thereby Decrease ARDS and MOF

With our long-term interest in the pathogenesis of postinjury MOF,[29,30] our working hypothesis extended beyond reduced stored blood use during hospitalization.

We proposed that PolyHeme, in lieu of stored RBCs during initial resuscitation, would attenuate the adverse immunoinflammatory effects of allogeneic RBC transfusion and ultimately reduce the incidence of ARDS and MOF. Stored blood is reportedly safer than ever due to comprehensive screening for disease transmission, but the potential adverse effects of packed RBC storage on the immune response to injury and illness are becoming more apparent.[31,32] We have been interested in the proinflammatory effects of stored RBCs and, specifically, their capacity to provoke polymorphonuclear neutrophil (PMN) cytotoxicity. PMN is a key cellular mediator in the pathogenesis of postinjury MOF. Consequently, PMN functional responses are evaluated as a clinical surrogate for the 2-event model of MOF; *i.e.,* inflammatory priming and subsequent activation. The 2-event construct of postinjury MOF is based on the fundamental concept that injury primes the innate immune system such that a second insult, during this vulnerable window, provokes unbridled systemic inflammation resulting in organ dysfunction.[33] Priming is defined as an enhanced response to a stimulus that is due to prior exposure of the cell to a different agonist.[34] In our ongoing epidemiologic studies,[35] we have shown that more than 6 units of RBC transfusion within the first 12 hours postinjury is an independent risk factor for MOF.[36] Furthermore, the age of transfused blood within the first 6 hours postinjury correlates with the incidence of MOF.[37] Previous studies in our center have shown that after severe injury, patients at high risk for MOF have circulating PMNs that are primed for cytotoxicity within the first 6 hours postinjury, as marked by the increased surface expression CD11b/CD18, p 38 mitogen-activated protein kinase (MAPK) activation, release of cytotoxic products in response to formyl-methionyl-leucyl-phenylalanine (fMLP), and delayed apoptosis.[38]

The precise mechanisms linking packed RBC transfusion and PMN priming remain to be established, but many believe that passenger leukocytes accompanying RBCs in storage are important in the generation of proinflammatory agents.[39] Plasma from stored RBCs primes PMNs in vitro, and this effect has been shown to increase progressively from 14 to 42 days of storage.[40] Some investigators have incriminated cytokines (TNF-a, IL_1, IL_6, IL_8 and IL_{18}) generated during storage[41] while we have focused on proinflammatory lipids presumably generated from the RBC membrane.[42] Metabolites of the arachidonic acid cascade have been strongly implicated in the pathogenesis of transfusion-related acute lung injury.[43] Although prestorage leukoreduction of RBCs decreases the generation of cytokines, this process does not eliminate PMN priming.[44] Thus, collectively, these studies suggest a blood substitute devoid of proinflammatory agents will avoid the immunomodulatory consequences of allogeneic RBCs.

In preparation for clinical trials, we conducted in vitro and in vivo studies to test our hypothesis that PolyHeme—free of inflammatory cytokines and lipids—would eliminate the PMN priming previously documented with stored RBCs and translate to reduced ARDS and MOF.[45] Human PMNs were isolated from healthy volunteers, and the plasma fraction was separated from packed RBCs at 42 days of storage in our blood bank (the last day stored RBCs can be transfused clinically but often the first RBCs infused into trauma patients).[46] The isolated PMNs were incubated with either RBC plasma or PolyHeme at concentrations calculated to be equivalent of up to 8 units of transfusion. The plasma fraction representing 3 or more units of stored RBCs primed the human PMNs for enhanced superoxide production and elastase release (Fig. 1).

We further tested our hypothesis in an established two-event model of MOF, *i.e.,* trauma/hemorrhagic shock as a priming event followed by toll-like receptor 4 (TLR4) engagement as an activating event.[47] Our primary study objective was to contrast HBOC versus crystalloid in the prehospital phase, but we expanded the study groups to encompass the possible availability of stored blood in the field and our previous in-hospital phase II clinical work with HBOC resuscitation. We selected acute lung injury as our primary study endpoint because ARDS is the first manifestation of postinjury MOF. Rats underwent laparotomy and hemorrhagic shock (30 mm Hg $\times$ 45 minutes) and were resuscitated over 2 hours in a clinically relevant design; *i.e.,* 2$\times$ volume of shed blood (SB) using NS in the first 30 minutes; half volume of SB in the next 30 minutes; another 2$\times$ SB volume with NS over the remaining 60 minutes. Study groups represented alternative fluid strategies during the first hour of resuscitation: (1) in-hospital SB (standard resuscitation), (2) in-hospital HBOC, (3) prehospital SB, and (4) prehospital HBOC. Global physiologic response was assessed via tissue oxygenation [near-infrared (NIR) spectroscopy] and arterial base deficit (BD), and pulmonary response via lung PMN accumulation and vascular permeability. Prehospital HBOC resuscitation provided the most efficient recovery of tissue oxygenation, (Fig. 2) and correction of BD had the greatest reduction in pulmonary PMN accumulation and abrogated acute lung injury (Fig. 3). The findings in this controlled in vivo study further supported our hypothesis that initial HBOC resuscitation attenuates the postshock inflammatory response and secondary organ dysfunction.

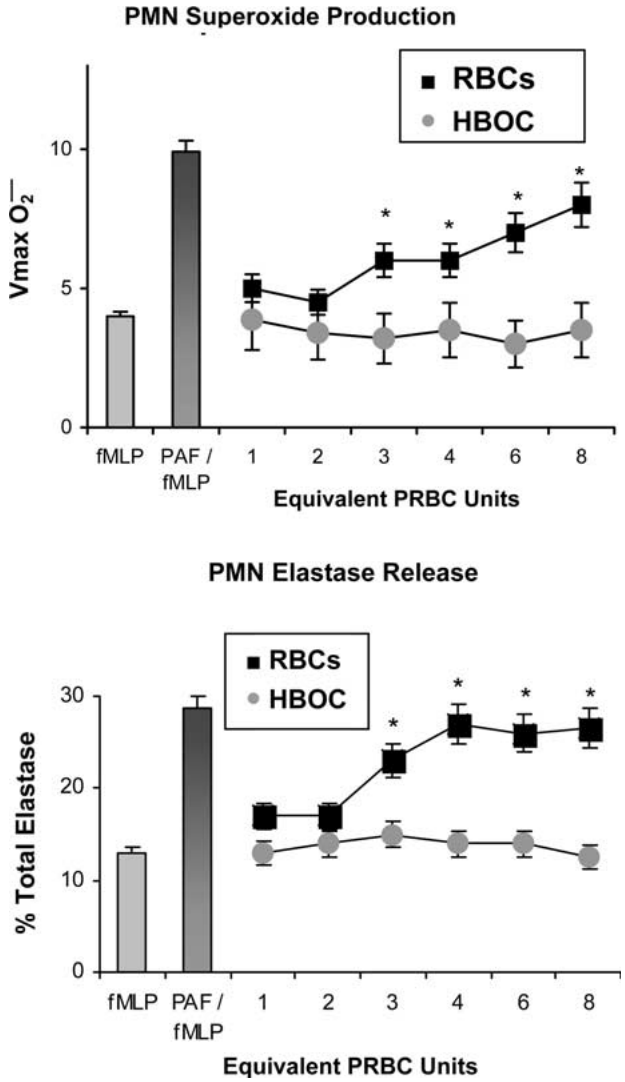

Figure 1. Isolated human polymorphonuclear neutrophils (PMNs) were incubated with either the plasma fraction from stored red blood cells RBCs or PolyHeme at concentrations equivalent to 1–8 units of acute transfusion. **A.** PMN superoxide production. **B.** PMN elastase release. Formyl-methionyl-leucyl-phenylalanine (fMLP) is employed as a PMN activator; and platelet-activating factor (PAF) (primer) followed by fMLP approximates maximal PMN response. *$P < 0.05$.

In our subsequent clinical trial, injured patients requiring urgent transfusion were administered either PolyHeme (up to 20 units = 1,000 g) or stored RBCs for their initial 12 hour of resuscitation.[48] PMN priming was determined by the surface expression of CD11b/CD18 in whole blood and superoxide production in isolated PMNs. The study groups [stored RBC (n = 10) vs. PolyHeme (n = 9)] were comparable with respect to injury severity (ISS = 27.9 ± 4.5 vs. 21.9 ± 2.7), physiologic compromise (emergency department pH = 7.22 ± 0.04 vs. 7.19 ± 0.08), and Hb transfusion in the first 24 hours (units = 14.1 ± 2.0 vs. 14.5 ± 1). Circulating PMNs from patients resuscitated with stored RBCs manifested evidence of priming through increased CD11b/CD18 expression and enhanced superoxide production (Fig. 4). Three patients (30%) in the stored RBC group died of MOF while all patients in the PolyHeme group survived.

To further investigate the impact of early resuscitation with PolyHeme in lieu of stored RBCs, we extended our clinical trial to evaluate the systemic levels of proinflammatory cytokines (IL_6, IL_8), counterregulatory cytokines (IL_{10}, IL_{11}), and markers of endothelial activation [soluble intercellular adhesion molecule (sICAM), soluble E selectin (sE-selectin)].[45] The study groups [stored RBC (n = 7) vs. PolyHeme (n = 18)] were comparable with respect to injury severity. Patients resuscitated with stored RBCs had higher levels of proinflammatory cytokines IL_6 and IL_8 and higher levels of counterregulatory cytokine IL_{10} (Fig. 5), with a trend toward higher sICAM, and sE-selectin levels. We have not enrolled a sufficient number of injured patients to definitively address the ultimate study objective—reduction of postinjury MOF. However, the incidence of MOF in acutely injured patients given PolyHeme during their initial resuscitation for whom we had complete data (n = 20) was 15% contrasted with a predicted incidence of 37% ($P < 0.05$) based on our MOF prediction model.[45] In sum, these clinical trials in trauma patients suggest that PolyHeme used in the early resuscitation of patients with hemorrhagic shock attenuates the immunodysfunction associated with stored RBC transfusion and thereby reduces the incidence of postinjury MOF.

Acute Hemorrhagic Shock: When Stored RBCs Are Unavailable in Trauma Care

The most compelling indication for HBOC is the scenario in which stored RBCs are unavailable. This potential benefit for military use has largely driven the development of HBOCs, but there are also a number of key applications in civilian trauma care. Most conspicuous is the role in prehospital care, particularly for extended transport times. But there are also remote hospitals throughout the country in which stored blood is simply not available or is rapidly depleted when multiple casualties are encountered. There have been well-designed animal models that strongly suggest prehospital low-volume resuscitation with HBOCs can save lives.[23–25] Despite the evidence, the scientific design and ethical conduct of clinical trials to establish efficacy of HBOCs when RBCs are unavailable remain a challenge.[49,50] To best approximate this scenario, we compared the 30-day mortality in 171 trauma patients given up to 20 units (1,000 g) of PolyHeme with a historic control of 300 surgical patients who refused

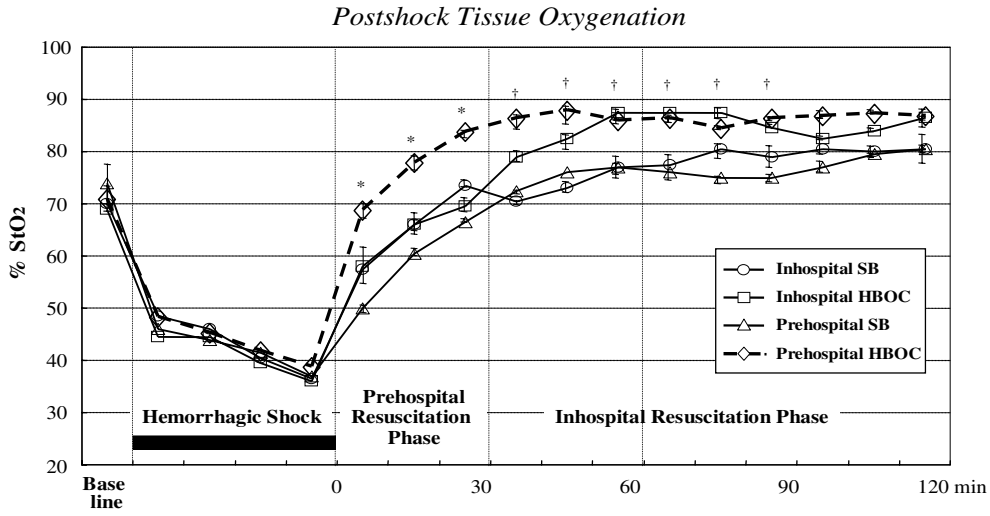

Figure 2. Tissue oxygenation (St02 = tissue oxygen saturation) was monitored continuously with a near-infrared spectroscopy device placed on the animal's hind limb. *$P < 0.05$ versus other groups + <0.05 prehospital hemoglobin-based oxygen carriers (HBOC) and in-hospital HBOC versus prehospital shed blood (SB) and In-hospital SB

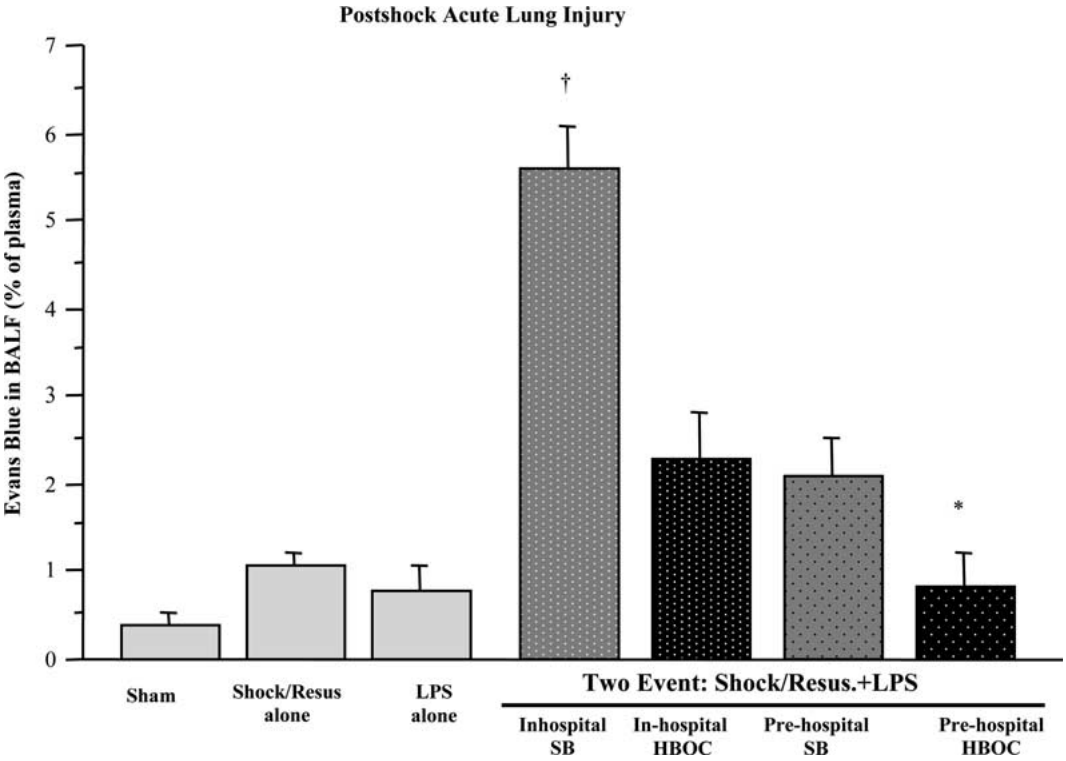

Figure 3. Acute lung injury, determined by Evans blue alveolar extravasation, was evaluated at the end of the study (8 hours postinsult). Simulated prehospital hemoglobin-based oxygen carriers (HBOC) resuscitated abrogated early ALI. *$P < 0.05$ versus In-hospital shed blood (SB). †$P < 0.05$ versus all other 2-event groups

stored RBCs on religious grounds.[51] The trauma patients received rapid infusion of 1–2 units (n = 45), 3 – 4 units (n = 45), 5–9 units (n = 47), or 10–20 units (n = 34) of PolyHeme; 40 patients had a nadir RBC Hb ≤ 3 g% (mean = 1.5 $\pm$ 0.7 g%). Total Hb was adequately maintained (mean = 6.8 $\pm$ 1.2 g%) via plasma Hb added by PolyHeme. The 30-day mortality was 25.0% (10 of 40 patients) compared with 64.5% (20 of 31 patients) in control patients (Fig. 6).

A personal experience with PolyHeme during our in-hospital FDA-approved phase II studies has convinced me the time has arrived for licensing of HBOCs for trauma care.[45] An 18-year-old man arrived by ground ambulance at our emergency department in extremis after a gunshot wound to the abdomen from a high-velocity elk-hunting rifle (30.06, hollow soft point 220 gr, muzzle energy 2,840 ft/lb). Because of immediate availability, 10 units of PolyHeme (maximum dose permitted at the time) were administered during the first 14 minutes of in-hospital resuscitation, representing greater than 91% of total circulating Hb at end infusion (RBC Hb = 0.7 g%). The missile entered the left midabdomen and exited posteriorly. At laparotomy, we encountered an avulsed shattered left kidney with secondary aortic and vena caval perfora-

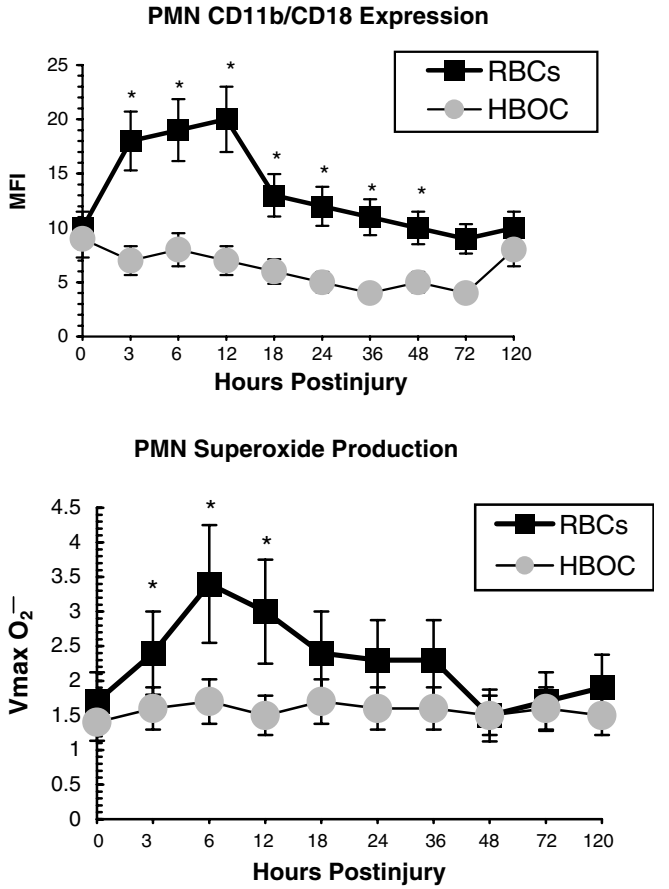

Figure 4. Circulating polymorphonuclear neutrophils (PMNs) from injured patients who underwent initial resuscitation with either stored red blood cells (RBCs) or PolyHeme. **A**. PMN CD11b/CD18 receptor expression in whole blood. **B**. PMN superoxide production in isolated cells. **P* <0.05

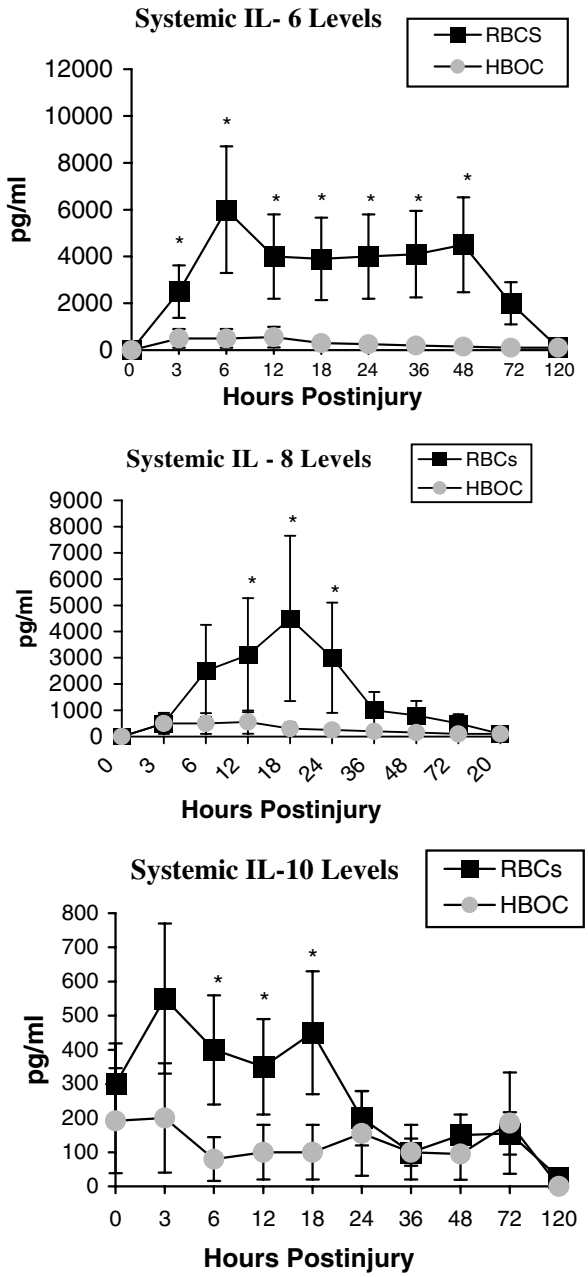

Figure 5. Systemic interleukin $(IL)_6$, IL_8, and IL_{10} from injured patients who underwent initial resuscitation with either stored red blood cells (RBCs) or PolyHeme. **A.** IL_6. **B.** IL_8. **C.** IL_{10}. **P* < 0.05.

tions, a partially transected superior mesenteric vein, and destructive injuries to his distal duodenum, proximal jejunum, midileum, and descending and sigmoid colon. In addition, he had massive soft tissue loss in the retroperitoneum, including the psoas and paraspinous muscles, and suffered a concussive spinal cord lesion with resultant paraplegia. The patient received an additional 40 units of packed RBCs during initial laparotomy, but ultimately, this gentleman survived to discharge without organ failure. We believe the immediate infusion of HBOC was pivotal in maintaining sufficient O_2 delivery during the critical period of massive blood loss to save this man's life.

CURRENT PHASE III US MULTICENTER PREHOSPITAL HBOC TRIAL

The optimal resuscitation fluid for acute blood loss remains unclear, and the practical options for prehospital care have been limited to expansion of the circulating blood volume. The issue is magnified in the combat scenario where access to blood transfusion is further delayed.[52] Resurgent interest in defining optimal field resuscitation has challenged the long-standing practice of unbridled crystalloid loading[53], citing the potential risk of exacerbating hemorrhage via dislodging hemostatic clots[54] and diluting plasma coagulation factors. Conversely, the magnitude of oxygen debt following hemorrhagic shock correlates directly with adverse

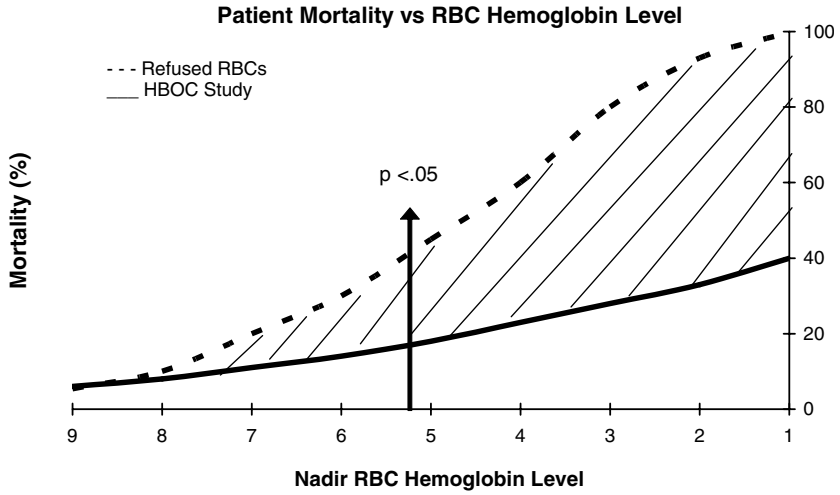

Figure 6. The 30-day mortality is compared in surgical patients who refused stored red blood cell (RBC) transfusion versus injured patients who were initially resuscitated with PolyHeme. The computer-generated curves are based on nadir hemoglobin (Hb) levels. Mortality was significantly less (P <0.05) in the PolyHeme group when RBC Hb was ≤ 5.3 g%, *i.e.,* critical anemia

Table 4.
Exception from informed consent requirements for emergency research in the USA

1. Human subjects are in life-threatening situation; available treatments are unsatisfactory.
2. Obtaining informed consent is not feasible.
3. Participation in the research holds out the prospect of direct benefit to the subjects.
4. The clinical investigation could not practicably be carried out without the waiver.
5. The investigational plan defines the length of the potential therapeutic window; investigator has committed to attempting to contact a legally authorized representative during that window.
6. The institutional research board has approved the informed consent document and procedures.
7. Additional protection of the rights and welfare of the subjects include: community consultation, public disclosure, establishment of an independent data monitoring committee, and consent to continue the study is obtained from the patient as soon as possible.

outcome.[55–57] The availability of HBOCs offers a new strategy for this clinical ''catch 22.'' Consequently, with this background and preliminary data, we initiated a multicenter prehospital trial in the US in January 2003. Severely injured patients, blunt or penetrating, with a SBP ≤ 90 mmHg due to acute blood loss are randomized at the scene to receive either the standard crystalloid resuscitation or PolyHeme. In the hospital, for the initial 12 hours postinjury, the control group receives stored RBCs as needed while the study group is administered PolyHeme up to 6 units and then stored RBCs as needed. The primary study endpoint is 30-day mortality; the secondary endpoints include amount of stored RBC transfusion, uncrossmatched RBC administration, incidence of circulation Hb <5 g%, and incidence of ARDS and MOF. The study is conducted, by necessity, with exception to informed consent.[58] By definition, critically injured patients are unable to provide informed consent and, due to the exigent circumstance, legal guardians or next of kin are often not accessible or appropriate for proxy consent during the narrow therapeutic window. One of the recent major advances in trauma research is the FDA codification of ''Exception from Informed Consent Requirements for Emergency Research'' detailed in the Code of Federal Regulation, Title 21, part 50, section 24 (21CFR 50.24), which became effective 1 November 1996 [Department of Health and Human Services (DHHS)/FDA, 1996]. The 7 fundamental components of this regulation are outlined in Table 4. Research protocols using this exception from informed consent must be conducted under a separate investigational new drug (IND) application to the FDA. The study population in the multicenter prehospital trial is targeted for 720 patients. As of this writing, 32 trauma centers throughout the US are enrolling patients, and the study remains unchanged after the final of four planned interim analyses (60–120–250–500–720 patients) by an independent data safety monitoring committee. We anticipate completing this prehospital trial in early 2006.

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REFERENCES

1. Department of Health and Human Services: 21 CFR601.25(d) (2).
2. Center for Biologics Evaluation and ResearchPoints to consider in the safety evaluation of hemoglobin-based oxygen carriers. Transfusion 1991;31:369–371.
3. Alayash Al. Oxygen therapeutics—Can we tame hemoglobin? Nat Rev Drug Discov. 2004;3:152–159.
4. Creteur J, Sibbald W, Vincent JL. Hemoglobin solutions—Not just red blood cell substitutes. Crit care Med 2000;28:3025–3034.
5. McFaul SJ, Bowman PD, Villa VM. Hemoglobin stimulates the release of proinflammatory cytokines from leukocytes in whole blood. J Lab Clin Med 2000;135:263–269.
6. Winslow RM. Current status of blood substitute research—towards a new paradigm. J Intern Med 2003;253: 508–517.
7. Center for Biologics Evaluation and ResearchPoints to consider on efficacy evaluation of Hemoglobin and perfluorocarbon based oxygen carriers. Transfusion 1994;34: 712–713.
8. Sloan EP, Koenigsberg M, Gens D, *et al.* Diaspirin cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock—A randomized controlled efficacy trial. JAMA 1999;282:1857–1864.
9. Hess JR, MacDonald VW, Brinkley WW. Systemic and pulmonary hypertension after resuscitation with cell-free hemoglobin. J Appl Physiol 1993;74:1769–1778.
10. Poli de Figueiredo LF, Mathru M, *et al.* Pulmonary hypertension and systemic vasoconstriction may offset the benefits of acellular hemoglobin blood substitutes. J Trauma 1997;42:847–856.
11. Gulati A, Sen AP, Sharma Ac, *et al.* Role of ET and NO in resuscitative effect of diaspirin cross-linked hemoglobin after hemorrhage in rat. Am J Physiol 1997;273:H827–H836.
12. Schultz SC, Grady B, Cole F, *et al.* A role for endothelin and nitric oxide in the pressor response to diaspirin cross-linked hemoglobin. J Lab Clin Med 1993;122:301–308.
13. Rohlfs RJ, Bruner E, Chiu A, *et al.* Arterial blood pressure responses to cell-free hemoglobin solutions and the reaction with nitric oxide. J Biol Chem 1998;273:12128–12134.
14. Boura C, Caron A, Longrois D, *et al.* Volume expansion with modified hemoglobin solution, colloids, or crystalloid after hemorrhagic shock in rabbits: effects in skeletal muscle oxygen pressure and use versus arterial blood velocity and resistance. Shock 2003;19:176–182.
15. Wettstein R, Cabrales P, Erni D, *et al.* Resuscitation from hemorrhagic shock with MalPEG-albumin: comparison with MalPEG-hemoglobin. Shock 2004;22:351–357.
16. Fushitan K, Imai K, Riggs AF. Oxygen properties of hemoglobin form the earthworm Lumbricus terrestric. J Biol Chem 1986;261:8414–8423.
17. Hirsch RE, Jelicks LA, Wittenberg BA, *et al.* A first evaluation of the natural high molecular weight polymeric lumbricus terrestric hemoglobin as an oxygen carrier. Art Cells Blood Subs Immob Biotech 1997;25:429–444.
18. Levy JH, Goodnough LT, Greilich PE, *et al.* Polymerized bovine hemoglobin solution as a replacement for allogeneic red blood cell transfusion after cardiac surgery: results of a randomized, double-blind trial. J Thorac Cardiovasc Surg 2002;124:35–42.
19. LaMuraglia GM, O'Hara PJ, Baker WH, *et al.* The reduction of the allogenic transfusion requirement in aortic surgery with hemoglobin-based solution. J Vasc Surg 2000;31:299–308.
20. Standl T, Burmeister MA, Horn EP, *et al.* Bovine haemoglobin-based oxygen carrier for patients undergoing haemodilution before liver resection. Br J Anaesth 1998; 80:189–194.
21. Kasper SM, Walter M, Grune F, *et al.* Effects of a hemoglobin-based oxygen carrier (HBOC-201) on hemodynamics and oxygen transport in patients undergoing preoperative hemodilution for elective abdominal aortic surgery. Anesth Analg 1996;83:921–927.
22. Hughes GS Jr, Antal EJ, Locker PK, *et al.* Physiology and pharmacokinetics of a novel hemoglobin-based oxygen carrier in humans. Crit Care Med 1996;24:756–764.
23. Handrigan MT, Bentley TB, Oliver JD, *et al.* Choice of fluid influences outcome in prolonged hypotensive resuscitation after hemorrhage in awake rats. Shock 2005;23: 337–343.
24. Manning JE, Katz LM, Brownstein MR, *et al.* Bovine hemoglobin-based oxygen carrier (HBOC-201) for resuscitation of uncontrolled, exsanguinating liver injury in swine. Shock 2000;13:152–159.
25. McNeil JD, Smith DL, Jenkins DH, *et al.* Hypotensive resuscitation using an polymerized bovine-based oxygen carrying solution leads to reversal of anaerobic metabolism. J Trauma 2001;50:1063–1075.
26. Gould SA, Moore EE, Moore FA, *et al.* Clinical utility of human polymerized hemoglobin as a blood substitute after acute trauma and urgent surgery. J Trauma 1997;43:325–332.
27. Johnson JL, Moore EE, Offner PJ, *et al.* Resuscitation of the injured patient with polymerized stroma-free hemoglobin does not produce systemic or pulmonary hypertension. Am J Surg 1998;176:612–617.
28. Gould SA, Moore EE, Hoyt DB, *et al.* The first randomized trial of human polymerized hemoglobin as a blood substitute in acute trauma and emergent surgery. J Am Coll Surg 1998;187:113–122.
29. Botha AJ, Moore FA, Moore EE, *et al.* Postinjury neutrophil priming and activation—An early vulnerable window. Surgery 1995;118:35–365.
30. Ciesla DJ, Moore EE, Johnson JL, *et al.* A 12 year prospective study of postinjury multiple organ failure. Arch Surg 2005;140:432–440.

31. Aiboshi J, Moore EE, Ciesla DJ, *et al.* Blood transfusion and the two-insult model of postinjury multiple organ failure. Shock 2001;15:302–306.
32. Silliman CC, Moore EE, Johnson JL, *et al.* Transfusion of the injured patient: proceed with caution. Shock 2004;21:291–299.
33. Moore EE, Moore FA, Harken HA. et al. The two-event construct of postinjury multiple organ failure. Shock (in press).
34. Ingraham LM, Allen JM, Higgins CP, *et al.* Metabolic membrane and functional responses of human polymorphonuclear leukocytes to platelet activating factor. Blood 1982;59:1259–1266.
35. Sauaia A, Moore FA, Moore EE, *et al.* Early predictors of post-injury multiple organ failure. Arch Surg 1994;129: 38–45.
36. Moore FA, Moore EE, Sauaia A. Blood transfusion—An independent risk factor for postinjury multiple organ failure. Arch Surg 1997;132:620–625.
37. Zallen G, Offner PJ, Moore EE, *et al.* Age of transfused blood is an independent risk factor for post-injury multiple organ failure. Am J Surg 1999;178:570–572.
38. Biffl WL, Moore EE, Zallen G, *et al.* Neutrophils are primed for cytotoxicity and resist apoptosis in injured patients at risk for multiple organ failure. Surgery 1999;126: 198–202.
39. Bordin JO, Heddle NM, Blajchman MA. Biologic effects of leukocytes present in transfused cellular blood products. Blood 1994;84:1703–1721.
40. Nielsen HJ, Reimert CM, Pedersen AM, *et al.* Time-dependent spontaneous release of white cell and platelet derived bioactive substances from stored human blood. Transfusion 1996;36:960–965.
41. Shanwell A, Dristiansson M, Remberger M, *et al.* Generation of cytokines in red cell concentrates during storage is prevented by pre-storage white cell reduction. Transfusion 1997;36:678–684.
42. Silliman CC, Paterson AJ, Dickey WO, *et al.* The association of biologically active lipids with the development of transfusion-related acute lung injury. Transfusion 1997;37:719–726.
43. Silliman CC, Voelkel NF, Allard JD, *et al.* Plasma and lipids from stored packed red blood cells cause acute lung injury in an animal model. J Clin Invest 1998;101:1458–1467.
44. Biffl WL, Moore EE, Offner PJ, *et al.* Plasma from aged stored red blood cells delays neutrophil apoptosis and primes for cytotoxicity-abrogation by post storage washing but not prestorage leukoreduction. J Trauma 2001;50:426–432.
45. Moore EE. Blood substitutes: the future is now. J Am Coll Surg 2003;196:1–17.
46. Partrick DA, Moore EE, Barnett CC, *et al.* Human polymerized hemoglobin as a blood substitute avoids transfusion-induced PMN priming for superoxide and elastase release. Shock 1997;7:24.
47. Masuno T, Moore EE, Cheng AM, *et al.* Prehospital hemoglobin based oxygen carrier (HBOC) resuscitation presents acute lung injury. Surgery (in press).
48. Johnson JL, Moore EE, Offner PJ, *et al.* Resuscitation with a blood substitute abrogates pathologic postinjury neutrophil cytotoxic function. J Trauma 2004;50:449–456.
49. Huston P, Peterson R. Withholding proven treatment in clinical research. N Engl J Med 2001;345:912–913.
50. McRae AD, Weijer C. Lessons from everyday lives-amoral justification for acute care research. Crit Care Med 2002;30:1146–1151.
51. Gould SA, Moore EE, Hoyt DB, *et al.* The life-sustaining capacity of human polymerized hemoglobin when red cells may be available. J Am Coll Surg 2002;195:445–455.
52. Gawande A. Casualties of war-military care for the wounded from Iraq and Afghanistan. N Eng J Med 2004;351:2471–2478.
53. Bickell WH, Wall MJ, Pepe PE, *et al.* Immediate versus delayed fluid resuscitation for hypotensive patients with penetrating torso injuries. N Engl J Med 1994;331:2206–1109.
54. Sandeen JL, Coopes VG, Holcomb JB. Blood pressure at which rebleeding occurs after resuscitation in swine and aortic injury. J Trauma 2004;54:5440–5117.
55. Claridge JA, Schulman AM, Young JS. Improved resuscitation minimizes respiratory dysfunction and blunts interleukin - 6 and nuclear factor - kB activation after traumatic shock. Crit Care Med 2002;30:1815–1819.
56. Dunham CM, Siegel JH, Weitreter L, *et al.* Oxygen debt and metabolic academia as quantitative predictors of mortality and severity of the ischemic insult in hemorrhagic shock. Crit Care Med 1991;19:231–241.
57. Shoemaker WC, Appel PL, Kram HB. Tissue oxygen debt as a determinant of lethal and nonlethal postoperative organ failure. Crit Care Med 1988;16:1117–1120.
58. Department of Health and Human Services. 21 CFR 50.24.