The use of a hemoglobin-based oxygen-carrying solution (HBOC-201) for extracorporeal membrane oxygenation in a porcine model with acute respiratory distress syndrome

C. L. Henderson, MD; C. M. Anderson, MD; D. L. Sorrells, MD; B. J. Wilson, MD; E. J. Dick, DMV; R. J. DiGeronimo, MD

Objective: **To evaluate whether hemoglobin-based oxygen-carrying solution (HBOC)-201 (Biopure) is an effective alternative to donor blood for extracorporeal membrane oxygenation support in a porcine model of acute respiratory distress syndrome (ARDS).**

Design: **Randomized animal clinical trial.**

Setting: **Animal surgical research laboratory.**

Subjects: **Immature Yorkshire swine were assigned to one of three groups: 1, noninjured animals, donor porcine blood primed circuit; 2, ARDS-injured, HBOC-201 primed circuit; or 3, ARDS-injured, donor blood primed.**

Interventions: **ARDS injury was induced in groups 2 and 3 with oleic acid infusion before bypass. All animals were placed on full venoarterial extracorporeal membrane oxygenation support for 8 hrs.**

Measurements and Main Results: **Physiologic variables and laboratory samples were measured at baseline and hourly for 8 hrs. Data analysis consisted of repeated-measures analysis of variance with *post hoc* analysis. We found that 100% of animals survived on extracorporeal membrane oxygenation for the duration of the study period. HBOC-supported animals had comparable oxygen delivery to both donor blood groups. Mean pulmonary artery pressure, heart rate, and lactate concentrations were higher in the injury groups. Blood pressure was mildly increased in HBOC animals ($p < .05$ vs. control animals). Methemoglobin concentrations in the HBOC group were elevated and increased over time on extracorporeal membrane oxygenation ($p < .001$).**

Conclusions: **HBOC-201 appears to be an effective alternative circuit-priming agent for use during extracorporeal membrane oxygenation. HBOC offers the advantages of rapid availability and diminished donor blood cell exposure. The efficacy of HBOC in longer duration bypass, and its associated methemoglobinemia, need to be further investigated. (Pediatr Crit Care Med 2004; 5:384–390)**

KEY WORDS: hemoglobin-based oxygen-carrying solution-201; hemoglobin-based oxygen-carrying solution; extracorporeal membrane oxygenation; acute respiratory distress syndrome; blood substitute

Extracorporeal membrane oxygenation (ECMO) is an accepted treatment modality for the rescue of critically ill neonates and children with high predicted mortality rates. Currently, ECMO offers survival rates of 80–90% in the neonatal population and up to 50–60% in children with potentially reversible cardiopulmonary failure (1). Use of ECMO in children requires the priming of an extracorporeal circuit with a large volume of donated human blood. A typical neonatal circuit requires 2 units of blood for the initial circuit prime, and an infant may be exposed to as many as 10 additional units over the course of a typical ECMO run. Donor blood requirements in older children are substantially greater, given the use of larger circuits and longer average duration of ECMO.

Reliance on donor blood products creates many difficulties with ECMO. The initiation of ECMO support is often delayed by the need to cross-match donor blood, which can increase morbidity and mortality rates in critically ill patients. In an ECMO transport setting, such blood bank delays may be more clinically significant, adversely altering outcome in children awaiting urgent cannulation (2). The use of ECMO requires repeated exposures to blood products from multiple donors, resulting in a significantly increased risk of transfusion-related infections and reactions. An additional concern is the generation of a postbypass systemic inflammatory response resulting from the use of stored donor blood products, which complicates ECMO management. This occurs with the interaction of an artificial circuit with various elements of banked blood, predominantly neutrophils and the complement system, which activate during bypass resulting in a proinflammatory response (3–5).

Given these concerns, the availability of an effective blood substitute for use during ECMO would be advantageous and could minimize the problems and risks associated with standard circuit priming

From the Department of Pediatrics (CLH, BJW, RJD) and Department of Surgery (DLS), Wilford USAF Hall Medical Center, Lackland AFB, TX; the Department of Surgery (CMA), University of Texas Health Sciences Center, San Antonio, TX; and the Department of Pathobiology (EJD), 59th Clinical Research Squadron, Lackland AFB, TX.

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Address requests for reprints to: Major Cody L. Henderson, MD, 2200 Bergquist Dr. Suite 1, 859 MDOS/MMNP, Lackland Air Force Base, Texas 78236–5300. E-mail: cody.henderson@lackland.af.mil

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and transfusion practices. We have recently described the successful use of the acellular hemoglobin product, hemoglobin-based oxygen-carrying solution (HBOC)-201, during ECMO in a healthy porcine animal model. Animals in this study supported with HBOC-201 had similar oxygen delivery and hemodynamics compared with donor-blood animals over a 6-hr ECMO run (6).

HBOC-201 is oxygen therapeutic made of acellular glutaraldehyde-polymerized bovine hemoglobin in a modified lactated Ringer's solution. It is not blood-type specific. Bovine hemoglobin and human hemoglobin have clinically relevant differences. The oxygen affinity of HBOC-201 is lower (P_{50} 30–35 mm Hg) than human hemoglobin (P_{50} 28 mm Hg), favoring the release of oxygen at the tissue level. It offers oxygen delivery with less viscosity than traditional blood. HBOC-201 has an extended shelf life at room temperature, allowing it to be readily available for emergent bypass and transports.

Although initial pilot studies using HBOC-201 for ECMO have been encouraging, its ability to support oxygen delivery in an injured animal model with increased oxygen demand and consumption has not been studied. Our objective in the present study was to evaluate the ability of the modified acellular blood substitute HBOC-201 to support a critically ill animal while on ECMO.

MATERIALS AND METHODS

The Wilford Hall Medical Center Institutional Animal Care and Use Committee approved this study. All animals were used and cared for in compliance with Department of Defense Directive 3216.1 and Air Force Regulation 169-2, *The Use of Animals in DoD programs*, and National Institutes of Health publication 85-23, *Guide for the Care and Use of Laboratory Animals*. A total of 24 juvenile piglets (7.7–15 kg) were used. Animals were assigned to one of three ECMO study groups; group 1 (n = 8), noninjured animals supported on ECMO with donor blood primed circuits; group 2 (n = 8), injured animals supported on ECMO with HBOC-201 primed circuits; and group 3 (n = 8), injured animals supported on ECMO with donor blood primed circuits. In groups 2 and 3, an oleic acid infusion (0.2 mL/kg) was given into the main pulmonary artery over 30 mins to induce an acute respiratory distress syndrome (ARDS)-like injury before placement on ECMO (7–10). Blood primed animals (groups 1 and 3) received donor porcine whole blood obtained from adult donors. Each blood unit was prepared for ECMO use by adding 50 mL of Tham, 40 mL of 25% albumin, 200 units of heparin, and 300 mg of calcium gluconate. Each unit of HBOC-201 was prepared for circuit priming by adding 300 mg of calcium gluconate, 40 mL of 25% albumin, 10 mL of Tham, and 5 mL of D10W. All circuits were primed in a standard manner with carbon dioxide gas, normal saline, and albumin. For HBOC priming, 250 mL (approximately 30 g of bovine hemoglobin) of prepared HBOC-201 was added, whereas for blood priming, two units (approximately 30 g of porcine hemoglobin) of prepared donor whole blood was added to saline primed circuits.

Animal Preparation. Peripheral intravenous access was obtained and crystalloid fluids were run at maintenance rates. Animals were orally intubated and placed on volume-controlled conventional mechanical ventilation following sedation with mask isoflurane. Initial ventilator settings included 40% inspired oxygen, 10 breaths/min, positive end-expiratory pressure of 5 cm H_2O, and an adequate tidal volume (range, 10–15 mL/kg) to maintain normal end-tidal CO_2. Ventilator settings were not adjusted after onset of injury and initiation of ECMO. Continuous anesthesia was provided with isoflurane and fentanyl, and all animals remained paralyzed throughout the ECMO run. Instrumentation included a femoral artery catheter, left jugular pulmonary artery catheter, and venoarterial ECMO cannulae (right external jugular vein and right carotid artery). Heart rate, blood pressure, and core body temperature were continuously monitored and recorded. A Foley catheter was used to monitor hourly urine output.

ECMO Procedure. Animals were placed on ECMO following a minimum 30-min rest period after instrumentation. Extracorporeal support was initiated in the ARDS-injured groups when the following criteria were met: a) Pao_2 to <50 mm Hg; or b) severe cardiovascular compromise defined as bradycardia <60 beats/min and/or a mean arterial blood pressure <50% of baseline. A heparin bolus (100 units/kg) was given to each animal at the time of cannulation, and a continuous heparin infusion was begun to maintain anticoagulation. The heparin infusion rate was adjusted hourly to maintain activated clotting times between 180–220 secs while on ECMO. ECMO pump flow was advanced to 100 $mL \cdot kg^{-1} \cdot min^{-1}$ over 30 mins, and sweep gas flow and oxygen concentration were adjusted to maintain circuit arterial Pao_2 200–300 mm Hg and $Paco_2$ 35–45 mm Hg. Animals received normal saline (10 mL/kg per bolus) if required for volume support to maintain circuit pump flows at 100 $mL \cdot kg^{-1} \cdot min^{-1}$. Animals were transfused 1 unit of whole blood (blood groups) or HBOC-201 (HBOC group) for any hemoglobin value of <6.5 g/dL (i.e., blood-primed animals exclusively received donor blood, and HBOC animals received only HBOC, to maintain minimum hemoglobin concentrations).

Laboratory Measurements. Blood was obtained at baseline (postinstrumentation) and then hourly throughout the 8-hr study period on ECMO for blood gas analysis, complete blood count, plasma hemoglobin, electrolytes,

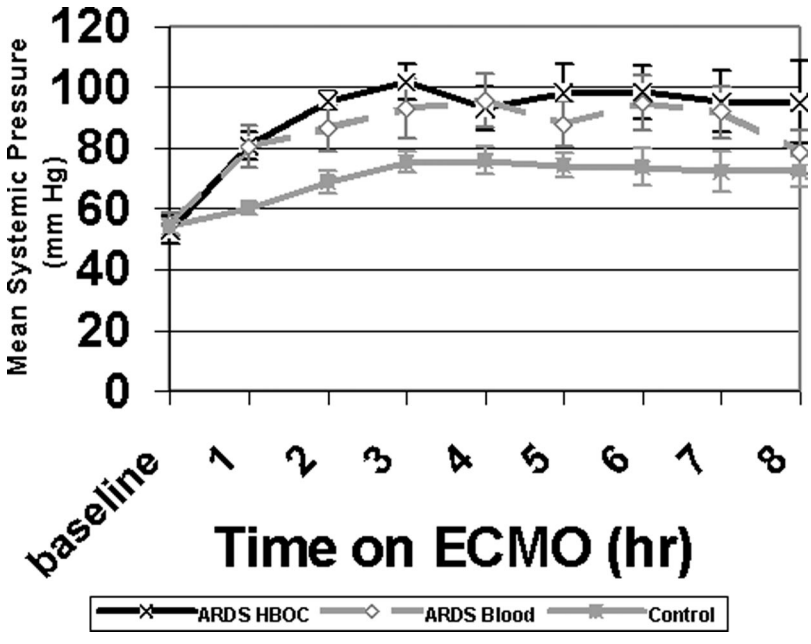

Figure 1. Mean arterial blood pressure. From 1 hr on, control trended lower than hemoglobin-based oxygen-carrying solution (*HBOC*; $p = .06$ for mean arterial pressure). There was no statistical difference between acute respiratory distress syndrome (*ARDS*) groups. *ECMO*, extracorporeal membrane oxygenation.

and blood lactate concentrations. Coagulation factors including prothrombin time and partial thrombin time were done at baseline and 1 and 8 hrs after starting ECMO.

Vital signs, mean pulmonary artery pressure, temperature, pulse oximetry, and mixed-venous oxygen saturation (Svo_2) were recorded every 15 mins. Urine output was recorded hourly. All animals were killed at the completion of 8 hrs on ECMO. Necropsy samples of heart, lungs, liver, and kidney were obtained and read by a veterinary pathologist.

Statistical Analysis. Data reported are mean ± SE for each group, unless otherwise stated. All statistical analyses were performed using a statistical software package for personal computers (SPSS, Windows version 11.0, SPSS, Chicago, IL). Analysis of variance for repeated measures was used. When significant interaction was detected, *post hoc* analyses were performed to compare the groups. We considered $p < .05$ to be statistically significant for all variables.

RESULTS

The body weight of the animals ranged from 7.7 to 15 kg, with HBOC piglets being slightly smaller (blood + ARDS 10.8 ± 1 kg, HBOC 9.9 ± 0.7 kg, control 12.2 ± 2 kg, $p <$ .05 HBOC vs. control). All 24 piglets survived the 8 hrs on ECMO. Pre-ECMO physiologic variables, arterial blood gases, and complete blood count values were similar in all groups.

In ARDS-injured animals, donor blood supported animals had greater blood transfusion requirements compared with the HBOC-primed group (6.2 ± 1.8 of donor blood vs. 3.1 ± 1.0 $mL \cdot kg^{-1} \cdot hr^{-1}$ of HBOC-201, respectively, $p < .05$) to maintain targeted hemoglobin concentrations. There was no transfusion difference between HBOC and noninjured control groups. Additionally, both injury groups required significantly more saline boluses for circuit support compared with noninjury animals (6.9 ± 0.9 $mL \cdot kg^{-1} \cdot hr^{-1}$ of saline boluses vs. 1.5 ± 0.3 $mL \cdot kg^{-1} \cdot hr^{-1}$, respectively, $p < .01$). There was no difference in the volume of blood sampled from any of the groups. Urine output was similar between all animals (9.9 ± 4.9 $mL \cdot kg^{-1} \cdot hr^{-1}$ for HBOC and 8.1 ± 4.3 $mL \cdot kg^{-1} \cdot hr^{-1}$ for blood injury groups, and 7.1 ± 5.8 $mL \cdot kg^{-1} \cdot hr^{-1}$ for noninjury control, $p > .05$).

Physiologic Data. Systemic arterial blood pressures were elevated in ARDS injury compared with control animals with the initiation of ECMO and remained elevated compared with baseline (Fig. 1). Heart rates were increased in all animals when placed on ECMO compared with baseline but were not significantly different between groups. Pulmonary artery pressures were increased in ARDS injury animals throughout the run but were not significantly different in HBOC vs. donor blood supported animals (Fig. 2).

Figure 3 shows mean Svo_2 values by group. Svo_2 levels in noninjured animals from hours 1–8 on ECMO were significantly higher compared with both ARDS groups ($p = .05$ vs. HBOC + ARDS, $p = .02$ vs. blood + ARDS).

Table 1 lists the results for blood gas data in all three groups. The arterial pH remained at or near baseline in each

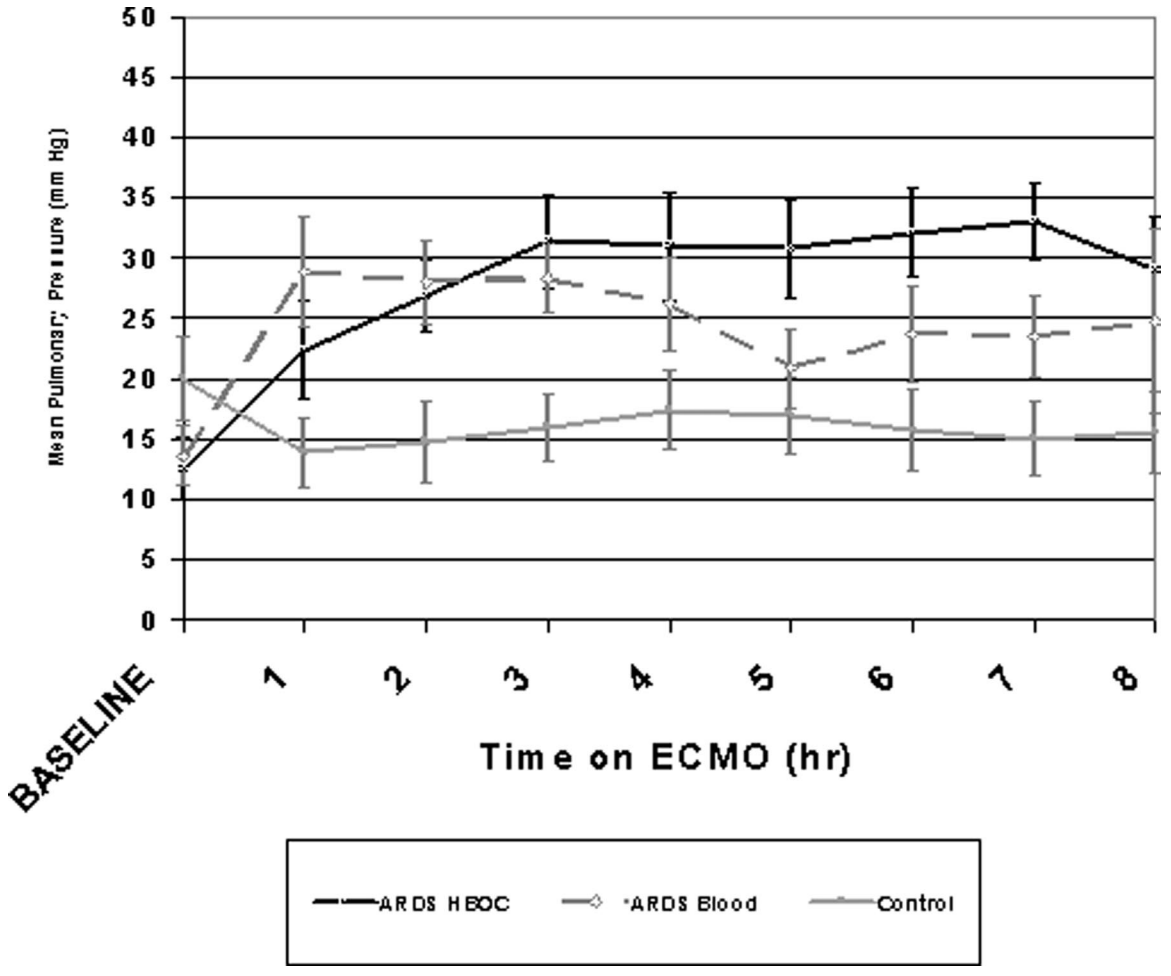

Figure 2. Mean pulmonary artery pressure. From 1 hr on, control was lower than group 2 ($p < .04$). There was no statistical difference between acute respiratory distress syndrome (*ARDS*) groups. *ECMO*, extracorporeal membrane oxygenation; *HBOC*, hemoglobin-based oxygen-carrying solution.

group. Although the pH in HBOC animals was slightly lower than both blood groups, it remained in the normal range throughout the 8 hrs on ECMO (>7.35). Whole blood lactate increased initially from baseline in both ARDS groups but returned to baseline by the end of the study period and was not different between injury groups. Arterial Pa_{O_2} values were higher in HBOC vs. ARDS blood group while on ECMO ($p < .02$).

Hematologic Data. Hemoglobin concentrations were similar in all three groups (Table 2). Once placed on ECMO, the hematocrit for the HBOC group declined sharply and remained below baseline compared with the blood groups. Plasma free hemoglobin concentrations averaged 2.6 ± 0.3 in HBOC animals compared with 0.1 ± 0.1 g/dL in both blood groups over the course of the study ($p < .001$). Platelet counts decreased in all groups on ECMO and continued to decline at later time points in HBOC animals ($p < .05$). No significant bleeding was noted in any animals. Activated clotting times were slightly higher in the HBOC animals (Table 2), and they required less heparin (HBOC received 48.5 ± 1.4 units·kg^{-1}·hr^{-1} vs. control at 57.1 ± 3.9 units·kg^{-1}·hr^{-1} and blood injury at 60 ± 2.8 units·kg^{-1}·hr^{-1}, $p < .05$).

Methemoglobin values are represented in Figure 4. Methemoglobinemia progressively increased during ECMO in HBOC animals compared with baseline ($p < .001$). Maximal values were seen at 8 hrs (5.7 ± 0.7%). The majority of HBOC-201 piglets reached approximately 5% methemoglobin, but one outlier reached 10% by 8 hrs of ECMO. Measured methemoglobin values in donor blood animals were negligible.

Pathology. Pathology evaluation showed more severe lung disease in both groups of ARDS injury animals than noninjury piglets. There was more necrosis, cellular infiltration, and airspace edema. HBOC-201 and blood ARDS groups were similar in the severity of their lung injury. Liver, heart, and kidney samples showed no gross pathologic or histology differences between injury groups. Five of eight HBOC piglets developed a purplish macular rash over the trunk within 1 hr of initiation of ECMO. None of these swine had hypotension or cardiovascular collapse associated with the rash. The rash subsequently resolved over several hours in all animals. Biopsy of affected skin revealed normal histology, with no inflammatory cell infiltrate or abnormal pigmentation.

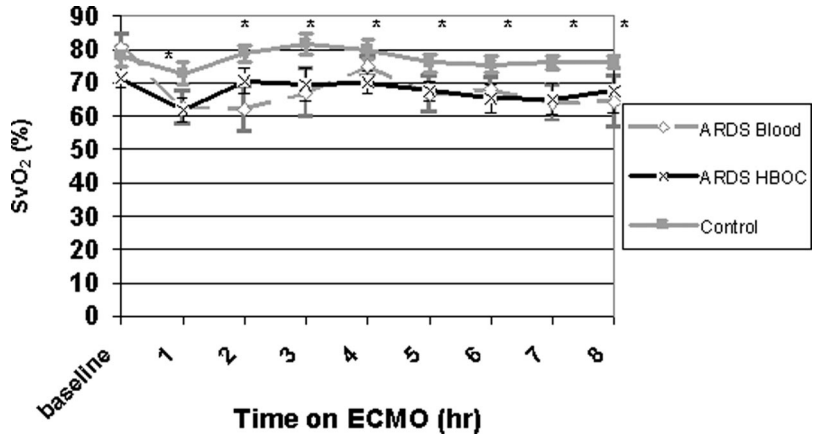

Figure 3. Mixed-venous oxygen saturation (Sv_{O_2}; %) by groups from baseline, pre-extracorporeal membrane oxygenation (*ECMO*) and pre-acute respiratory distress syndrome (*ARDS*) (0) until 8 hrs on ECMO. Control was greater (vs. ARDS + hemoglobin-based oxygen-carrying solution [*HBOC*] and ARDS + blood) from 1 to 8 hrs (*p = .05, .02 respectively).

Table 1. Blood gas data

Group	Time, hrs	Control	HBOC	Blood + ARDS
Lactate, mmol/L	Baseline	1.9 ± 0.2	2.8 ± 0.8	2.3 ± 0.3
	1	2.0 ± 0.2	3.6 ± 0.9[a]	4.0 ± 0.5[a]
	4	1.1 ± 0.1	1.8 ± 0.4	1.4 ± 0.2
	8	0.8 ± 0.1	2.0 ± 0.8[a]	2.0 ± 0.8[a]
Pa_{O_2}, mm Hg	Baseline	229 ± 42	205 ± 36	239 ± 41
	1	213 ± 9	197 ± 39[b]	125 ± 21[a]
	4	191 ± 11	214 ± 25[b]	142 ± 21[a]
	8	181 ± 25	255 ± 20[a,b]	177 ± 35
Pa_{CO_2}, mm Hg	Baseline	42 ± 2	39 ± 1	41 ± 2
	1	37 ± 1	40 ± 4	40 ± 4
	4	38 ± 2	43 ± 3[b]	37 ± 1
	8	40 ± 3	41 ± 2	39 ± 2
pH	Baseline	7.41 ± 0.02	7.40 ± 0.02	7.43 ± 0.03
	1	7.47 ± 0.02	7.35 ± 0.06[a]	7.41 ± 0.03[a]
	4	7.46 ± 0.02	7.38 ± 0.03[a]	7.49 ± 0.03
	8	7.43 ± 0.04	7.39 ± 0.02[a]	7.42 ± 0.06

HBOC, hemoglobin-based oxygen-carrying solution; ARDS, acute respiratory distress syndrome.
[a]$p < .05$, compared with control at the same time point; [b]$p < .05$, compared with blood plus ARDS at the same time point.

DISCUSSION

This study is the first to report the utility of the blood substitute HBOC-201 as a circuit-priming agent for use during ECMO in an ARDS animal model. HBOC-201 was able to successfully support these animals on ECMO for the duration of the study despite their increased metabolic oxygen demand. In ARDS-injured animals, both Sv_{O_2} and serum lactate concentrations were similar with HBOC and blood prime on ECMO, suggesting equivalent adequate oxygen delivery. In addition, the use of HBOC-201 priming resulted in the need for less supplemental blood transfusion and anticoagulation over the course of the ECMO run.

Several recent studies investigating the use of blood substitutes with extracorporeal circuits have been published (6, 11, 12). Neragi-Miandoab (11) reported the successful use of HBOC-201 for cardiac bypass in canines with a hematocrit dilution 60% of baseline. As previously referenced, work in our own laboratory demonstrated the utility of HBOC-201 for use in ECMO to support a healthy porcine model (6). HBOC-201 circuit priming was well tolerated resulting in good oxygen delivery despite relatively low hematocrits and less supplemental volume to maintain hemodynamic stability. In our present study, the use of HBOC-201 resulted in nearly a 50% reduction in transfusion volume when compared with whole blood over 8 hrs of ECMO support. Such a dramatic reduction in blood vol-

ume requirements over the course of a standard ECMO run would substantially reduce inherent infectious risks and potentially attenuate the inflammatory response resulting from the addition of stored blood products to the circuit.

Table 2. Hematology data

	Group	Control	HBOC + ARDS	Blood + ARDS
Hemoglobin, g/dL	Baseline	7.0 ± 0.2	7.5 ± 0.3	7.8 ± 0.4
	1	6.1 ± 0.3	6.2 ± 0.2	6.3 ± 0.3
	4	6.6 ± 0.3	6.4 ± 0.2	7.4 ± 0.4
	8	7.1 ± 0.4	6.2 ± 0.2	6.6 ± 0.4
Platelets, ×1000	Baseline	497 ± 93	349 ± 34[a]	371 ± 61
	1	394 ± 45	257 ± 19[a]	275 ± 30[a]
	4	308 ± 25	208 ± 26[a,b]	277 ± 27
	8	341 ± 62	177 ± 23[a,b]	295 ± 29
White blood cells, ×1000	Baseline	13.8 ± 1.4	12.3 ± 1.4	14.5 ± 2.0
	1	10.4 ± 0.8	7.3 ± 0.8[a,b]	10.4 ± 1.7
	4	12.0 ± 0.8	10.4 ± 1.5	13.0 ± 1.8
	8	12.6 ± 1.3	13.4 ± 2.6	14.3 ± 2.3
Activated clotting time, secs	1	214 ± 22	206 ± 7	210 ± 12
	4	192 ± 10	227 ± 9[a,b]	201 ± 13
	8	209 ± 10	253 ± 9[a,b]	215 ± 8
PT, secs	Baseline	14.7 ± 0.3	14.6 ± 0.3	14.4 ± 0.3
	4	14.9 ± 0.4	18.3 ± 0.7[a,b]	15.6 ± 0.3
	8	14.9 ± 0.4	17.9 ± 0.6[a,b]	15.6 ± 0.6
PTT, secs	Baseline	77.7 ± 6.8	67.7 ± 4.3	73.5 ± 8.1
	4	79.3 ± 4.3	68.7 ± 2.8[a,b]	114.4 ± 5.2
	8	95.1 ± 5.0	87.0 ± 3.2	94.3 ± 6.0

HBOC, hemoglobin-based oxygen-carrying solution; ARDS, acute respiratory distress syndrome; PT, prothrombin time; PTT, partial thromboplastin time.

[a] $p < .05$, compared with control at the same time point; [b] $p < .05$, compared with blood plus ARDS at the same time point.

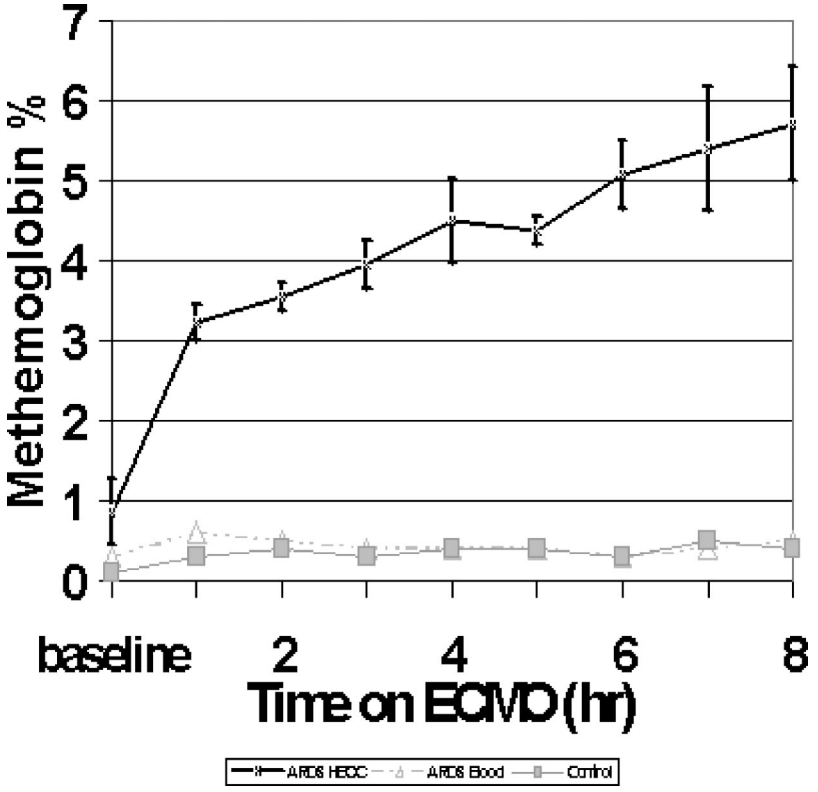

Figure 4. Methemoglobin (%) is shown from arterial blood gas analysis at baseline (0) and hours 1–8 on extracorporeal membrane oxygenation (*ECMO*). Hemoglobin-based oxygen-carrying solution (*HBOC*)-201 group is highest at all time points from 1 hr on (*$p < .001$). *ARDS*, acute respiratory distress syndrome.

HBOC-201 has been tested extensively as a low-volume oxygen delivery and trauma resuscitation medium in multiple animal models (11, 13, 14). In these studies, HBOC-201 has been shown to sustain tissue oxygenation. It is thought to preferentially increase oxygen delivery to ischemic areas (less viscous, acellular) while allowing for increased oxygen extraction at the tissue level (14). Its increased oncotic pressure (compared with blood and crystalloid) and vasopressor-like qualities make it an attractive alternative to other agents, especially in patients with hypovolemic and septic shock. HBOC-201 effectively increases vascular tone, much of which is likely due to nitric oxide and endothelin interactions with free hemoglobin (15–17).

In previous animal studies, HBOC-201 has been shown to transiently increase both pulmonary and systemic blood pressure. These reports have noted increases of systemic mean arterial pressures of 10–20 mm Hg, systemic vascular resistance of 70–120%, and pulmonary vascular resistance of 50– 70% with HBOC therapy (14, 15). We observed elevated systemic and pulmonary artery blood pressures on ECMO in ARDS animals compared with noninjured controls, without any significant differences noted between HBOC vs. donor blood priming. This most likely reflects a direct increase in systemic and pulmonary vascular resistance secondary to oleic acid injury, as well as the institution of venoarterial extracorporeal support itself. These factors may have masked any potential increased systemic and pulmonary artery pressure associated with HBOC transfusion as has been observed in previous studies (15–17).

The generalized vasopressor effect seen with the use of acellular blood substitutes such as HBOC-210 may be advantageous when treating hypovolemic shock. However, excessive vasoconstriction in the presence of severe hypovolemia may adversely effect the perfusion to the peripheral and splanchnic vasculature. Previous generation acellular hemoglobin solutions had a pronounced systemic vasoconstrictive response; however, more recent products such as HBOC-201 have shown a lessening of this effect and better support of end-organ oxygenation and perfusion (18–21). During ECMO, vs. conventional hypovolemic resuscitation, the potential concern over vasoconstriction may be less important given the significant improvement in per-

Hemoglobin-based oxygen-carrying solution-201 appears to be an effective alternative circuit-priming agent for use during extracorporeal membrane oxygenation.

fusion that usually occurs with the initiation of partial bypass.

All animals initially had similar hemoglobin, hematocrit, and platelet counts. As was seen with the previous study using healthy animals, injured HBOC pigs tolerated drastically lower hematocrits. Platelet counts traditionally decline with ECMO initiation, and this occurred in all groups in our study. HBOC-201-primed circuits before the initiation of ECMO were platelet free, whereas donor-blood primed circuits had platelets present. This most likely is the reason for a more precipitous decline in platelet numbers in the HBOC animals during ECMO. Despite this dilution of the platelets in the HBOC group, overall counts remained in the normal range and none of the animals in any of the groups developed any significant clinical bleeding.

In addition to its effects on platelet numbers, circuit volume dilution in association with HBOC priming may affect other aspects of the coagulation system as well. Prothrombin times were mildly prolonged in HBOC animals, which may be a result of endogenous factor dilution during ECMO. Alternatively, it is possible that the use of HBOC may result in an increased consumption or decreased production of platelets and coagulation factors, and HBOC-supported animals may ultimately may require more factor transfusions over longer ECMO runs than were done in our study.

HBOC animals required significantly less heparin to maintain anticoagulation compared with donor blood. This may be a consequence of the lack of procoagulation factors and platelets in HBOC-201 primed circuits. Given the variable amount of these factors found in donor blood, HBOC may allow for more straightforward and safer heparin dosing in patients at the initiation of extracorporeal bypass.

Dermatologic manifestations have been previously reported in the HBOC literature in both human and animal subjects (6, 22). Although such rashes have in general been noted to be self-resolving without associated morbidity, their occurrence needs to be closely monitored with future HBOC experiments. In human patients, skin rashes seen with HBOC have not been shown to involve an immunoglobulin G or immunoglobulin E mediated response (22). In the present study, consistent with the previous literature, rashes observed in HBOC animals were brief in character and not associated with any hemodynamic compromise or other apparent clinical morbidity. It is possible that skin manifestations seen in these animals may be related to a transient, vasoconstrictive response of the peripheral vascular beds with the initial exposure to HBOC; however, their etiology remain unclear and need to be further characterized.

Methemoglobinemia is the result of a failing balance of hemoglobin oxidation and reduction. Methemoglobin, the oxidized form of hemoglobin, is deficient in oxygen binding and impedes oxygen delivery. Methemoglobin reductase within erythrocytes provides the primary mechanism for reducing hemoglobin (11). It is possible that reduced absolute levels of methemoglobin reductase, with the hematocrit dilution found in HBOC-supported animals, resulted in increased methemoglobin concentrations. HBOC animals did develop significant methemoglobinemia that continued to progress through the duration of the study period. A similar pattern was described in our prior study with healthy pigs, where methemoglobinemia increased over the 6-hr study period (mean of 2.6 $\pm$ 1.3%) as well.

Other investigators working with acellular blood substitutes, including Neya et al. (12), have shown that methemoglobinemia occurs with *in vitro* priming of cardiopulmonary bypass circuits. This was greatly reduced by the addition of small volumes of red blood cells to the circuit, suggesting a need for some critical amount of intracellular methemoglobin reductase activity. Additional recent studies have suggested that acellular hemoglobin products may be more susceptible to methemoglobin conversion via interactions with endogenous nitric oxide and the vascular capillary beds (16, 17). Regardless of its etiology, the concentration of methemoglobinemia achieved in our study did not appear to adversely effect clinical oxygen delivery in HBOC-supported animals. However, further investigation into its potential clinical impact in a more prolonged ECMO run is needed.

CONCLUSIONS

In our ARDS porcine model, HBOC-201 appears to be an effective alternative to donor blood when used for ECMO circuit priming. HBOC appears to have equivalent efficacy to donor blood and may offer several potential advantages. The ready availability of an effective blood substitute with a long shelf half-life will avoid significant delays in obtaining blood. HBOC-201 essentially eliminates the risk of infectious complications, alloimmunization, and exposure to multiple donors. However, the most efficacious role for HBOC-201 with ECMO, including initial dosing volume and its subsequent use for circuit transfusion support, as well as potential concerns for methemoglobinemia and effects on platelet and coagulation factor consumption, remains to be clarified.

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