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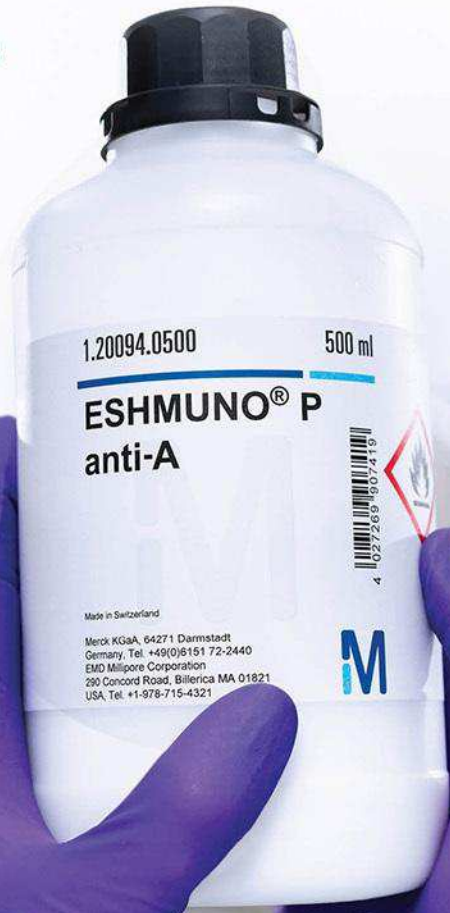

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Use of the blood substitute HBOC-201 in critically ill patients during sickle crisis: a three-case series

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BACKGROUND: Red blood cell (RBC) transfusion is an important treatment modality during severe sickle cell crisis (SCC). SCC patients who refuse, or cannot accept, RBCs present a unique challenge. Acellular hemoglobin (Hb)-based oxygen carriers (HBOCs) might be an alternative for critically ill patients in SCC with multiorgan failure due to life-threatening anemia. HBOC-201 (HbO2 Therapeutics) has been administered to more than 800 anemic patients in 22 clinical trials, but use of any HBOCs in critically ill sickle cell patients with organ failure is exceedingly rare. In the United States, HBOC-201 is currently only available for expanded access.

CASE REPORT: We report three cases of HBOC-201 administered to critically ill sickle cell disease patients in SCC with multiorgan failure, either who refused RBCs (Jehovah's Witnesses) or for whom compatible RBCs were not available.

RESULTS: Two patients received more than 20 units of HBOC-201, while the other received 6. The 27 units used in the third case equals the largest volume a patient has successfully received to date. All three patients survived to hospital discharge.

CONCLUSION: These reports suggest that blood substitutes such as HBOC-201 can provide an oxygen bridge in SCC with multiorgan failure, until corpuscular Hb levels recover to meet metabolic demand, and highlight the compelling biochemical properties that warrant further investigation.

The standard of care for sickle cell crisis (SCC) is largely diagnostic and supportive.[1,2] In cases where hemoglobin (Hb) levels are insufficient to fulfill metabolic needs, red blood cell (RBC) and exchange transfusion are often utilized. However, Jehovah's Witnesses (JWs) typically refuse blood products[1,3-5] or compatible RBCs may not be available or safe.[6] Cell-free Hb substitutes have improved over the past 30 years,[7,8] with significant improvements in the safety profiles and clinical outcomes of second-generation Hb-based oxygen carriers (HBOCs).[9] HBOC-201 is cell-free polymerized bovine Hb (Hb glutamer-250 [bovine]) with a P50 (oxygen pressure at which 50% of oxygen-binding sites are saturated) of 40 (±6) mmHg, compared to 27 mmHg for native human Hb, and concentration of 130 g/L, originally developed by Biopure Corporation. In the United States it is only available for expanded access.[10] HBOC-201 has been administered to more than 800

ABBREVIATIONS: 2,3-BPG = 2,3-bisphosphoglycerate; CXR = chest x-ray; HBOC(s) = hemoglobin-based oxygen carrier(s); JW(s) = Jehovah's Witness(-es); SCC = sickle cell crisis; SCD = sickle cell disease..

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patients with anemia from blood loss or sickle cell disease (SCD) in 22 clinical trials.[11] There are rare case reports of use of artificial oxygen carriers to treat sickle cell patients in crisis, including some with single or multisystem organ failure.[12,13] Here we present a series of three critically ill SCC patients who received HBOC-201 during the past 10 years, two JWs, and one for whom compatible RBCs were unavailable. In all cases, transfusion of blood was not an option.

CASE REPORTS

Consent was obtained from the patients in Cases 1 and 3. However, Case 2 occurred over a decade ago, and despite extensive efforts to find the patient, he could not be reached to obtain consent to use his data.

Case 1

A 30-year-old JW with SCD presented to Baystate Medical Center (Springfield, MA) with back pain, sore throat, and fever to 38.9°C. Admission labs revealed leukocytosis of 19.1 g/dL, Hb 7.7 g/dL, creatinine 0.8 mg/dL, and reticulocyte count 9.3%; the initial chest x-ray (CXR) was normal. He was treated with intravenous (IV) fluids and narcotics, but became hypotensive, tachycardic, and hypoxemic. Repeat CXR and labs revealed new infiltrates and worsening leukocytosis (32.2 g/dL), a Hb level of 5.2 g/dL with HbS of 80% on electrophoresis, and creatinine 2.4 mg/dL. Blood cultures grew coagulase-negative *Staphylococcus,* and the patient was diagnosed with acute chest syndrome secondary to *Staphylococcus* pneumonia. Despite early transfer to the medical intensive care unit and aggressive treatment with oxygen, IV fluids, and antibiotics, his respiratory, renal, hepatic, and neurologic function deteriorated. Hemodynamic monitoring revealed high-output heart failure with demand ischemia indicated by troponinemia and nonspecific T-wave changes on electrocardiogram. Metabolic demand was minimized with intubation and mechanical ventilation to allow chemical paralysis and cooling to normothermia. The number and volume of blood draws were minimized. Nevertheless, his Hb decreased to a nadir of 3.6 g/dL with progressive renal and liver failure, and his family and church agreed to HBOC administration. The Food and Drug Administration (FDA) approved the emergent use, and HbO2 Therapeutics donated HBOC-201. Following the Engelwood Protocol developed by Dr Shander (Clinical Trial NCT01881503),[14] the patient was given a total of 6 units of HBOC-201. The patient became hypertensive with the first unit, which resolved with a single dose of 10 mg IV labetalol. After 3 units, he developed methemoglobinemia to 10.2%, which resolved with ascorbic acid 500 mg IV BID for two doses, followed by 1000mg IV BID for seven doses. He was treated with 40,000 units of erythropoietin (EPO) every 72 hours for three doses, followed by 20,000 units every 12 hours for an additional five doses. Iron sucrose was also administered at 200 mg IV daily for 6 days, including two doses given for 1 day and a single additional 100-mg dose preceding this. His Hb (g/dL) increased to 4.2 after 1 unit, 4.5 after 3 units, and 5.0 after a total of 6 units (Fig. 1), and his renal and cardiac function significantly improved (Table 1). He was subsequently weaned from chemical paralysis and mechanical ventilation, after which he was transferred from the intensive care unit. His renal, hepatic, cardiac, and neurologic function all recovered, and he was discharged from the hospital after 32 days with a Hb level of 5.0 g/dL.

Case 2

A 28-year-old man with SCD presented to Methodist Hospital (Omaha, NE) with severe musculoskeletal pain consistent with SCC. At admission Hb level was 9.8 g/dL, reticulocyte count 15%, and pain was treated with narcotics. On Hospital Day 2, he became febrile to 38.9°C with respiratory failure and leukocytosis to 15.7 g/dL. CXR revealed bibasilar atelectasis, and he was started on empiric antibiotics. He was diagnosed with acute chest syndrome secondary to sepsis due to community-acquired pneumonia. By Day 4 his Hb level decreased to 8.6 g/dL, his pain escalated, and his heart rate increased to 155 with acute congestive heart failure, which was treated with IV lasix. He began receiving iron and EPO on Day 6. By Day 7 his Hb level decreased to a nadir of 3.7 g/dL and he developed ischemic changes on EKG and new hepatic failure. Repeat CXR revealed pneumonia, with negative blood and urine cultures. The patient agreed to RBC transfusion at this time, but no compatible RBCs could be located. Consequently, the FDA approved expanded access to HBOC-201. The patient received approximately 1.8 units/day over the next 2 weeks, for a total of 27 units, during which his Hb level increased to 6.4 g/dL (Fig. 1). This was accompanied by an increase in free Hb from 0.1 to 1.1 g/dL. Mean arterial pressure increased, occasionally as high as 95 mmHg. No treatment was given for hypertension. The patient's pain improved, and he was discharged on Hospital Day 22, with a Hb level of 5.7 g/dL.

Case 3

A 19-year-old JW with SCD presented to Levine Cancer Institute (Concord, NC) with diffuse musculoskeletal pain and dyspnea. Admission labs revealed a leukocytosis to 13.6×10^9/L and Hb level of 8.1 g/dL, with a normal chemistry profile and CXR. He was initially treated with IV fluids and narcotics. On Hospital Day 5, his dyspnea worsened with hypoxia to SaO_2 77%, and repeat CXR revealed a new right lower lobe infiltrate. His leukocytosis worsened to 21.5 and his Hb level decreased to 7.7 g/dL. The next day, he developed tachycardia to 122, fever to 39.4°C,

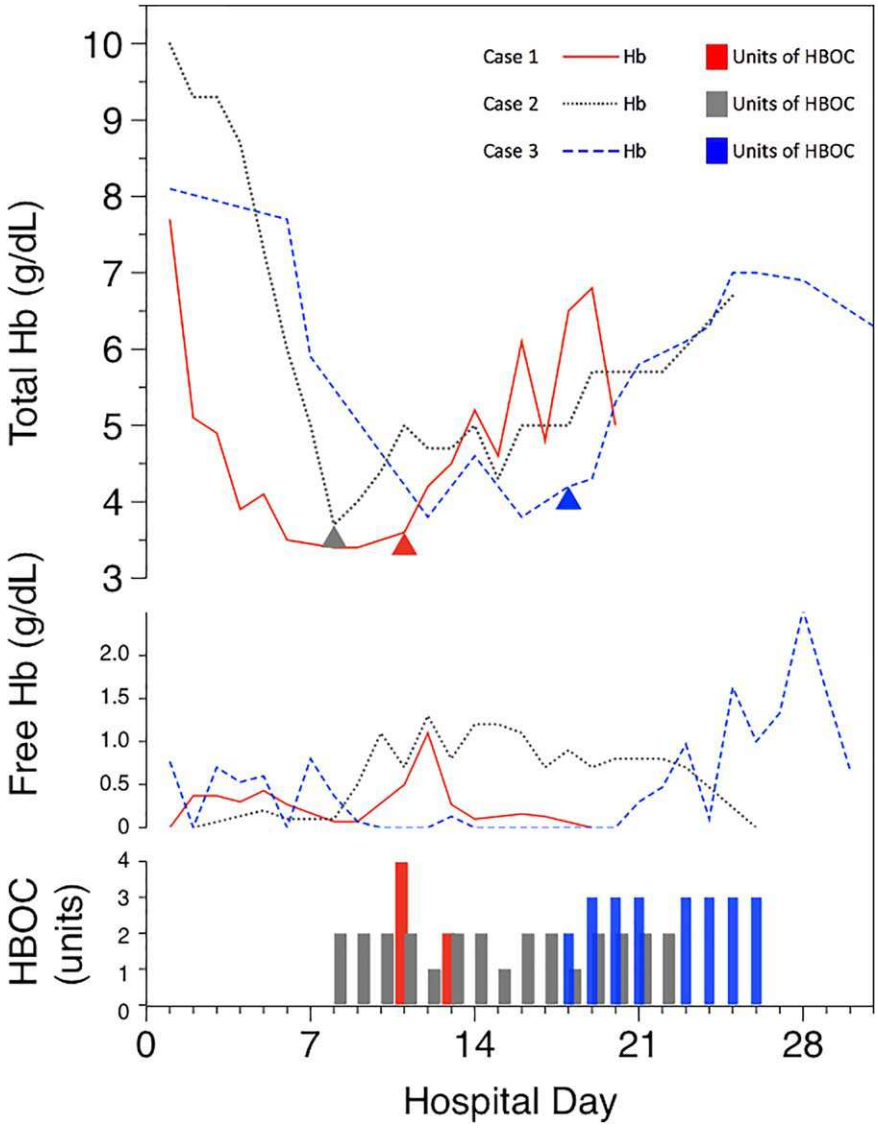

Fig. 1. Hb level, free Hb level, and units of HBOC transfused in three patients during days of hospital admission. Total and free Hb levels are shown for Cases 1 (red line), 2 (gray dotted line), and 3 (blue dashed line). The day of hospitalization on which the first unit of HBOC-201 was transfused is indicated on each curve by an arrowhead. The bars in the lower portion of the graph are color coded to each of the three cases and align with the day on which HBOC-201 was transfused, and the bar height correlates with number of units transfused on each day.

and a decrease in Hb level to 5.9 g/dL. He was diagnosed with sepsis and acute chest syndrome secondary to nosocomial pneumonia. Treatment included supplemental oxygen and empiric antibiotics, but the patient developed worsening respiratory failure and required subsequent escalation to noninvasive ventilation. He refused RBC transfusions and was supported with 40,000 units of EPO on Monday, Wednesday, and Friday; 1 mg of folic acid daily; 200 mg of iron sucrose IV for 5 days; and vitamin B12. His Hb level decreased to a nadir of 3.8 g/dL on Day 11, and IV iron was initiated at that time. His Hb level increased to 4.6 g/dL, before decreasing back to 3.8 g/dL on Day 15. At this point, the patient agreed to receive HBOC-201. The FDA and institutional review board emergently approved expanded access, and he received 23 units over 10 days. He developed a carboxyhemoglobinemia to 10.4% and a methemoglobinemia to 6%, which was treated with 1000 mg of oral ascorbic acid every 12 hours. His Hb level increased to 6.6 g/dL (Fig. 1), and his cardiac and pulmonary function improved. His course was complicated by pulmonary embolism and *Clostridium difficile* infection, both of which were successfully treated. He was discharged on Hospital Day 48 with a Hb level of 6.6.

TABLE 1. Hb, free Hb, creatinine, ALT, and lactate at three time points: hospital admission, before HBOC, and after HBOC

	Case	Hospital admission	Before HBOC	After HBOC
Hb (g/dL)	1	7.7	3.6	6.1
	2	10	3.7	6.7
	3	8.1	3.8	7
Free Hb	1	0	0	0.1
	2	NA	0.1	0.7
	3	0.77	0	1.33
Creatinine	1	0.8	5.9	2.9
	2	NL	NL	NL
	3		0.88	0.94
ALT	1	100	269	69
	2	150	175	125
	3		52	84
Lactate	1	4	0.7	0.6
	2			
	3		4.5	1.4

ALT = alanine aminotransferase; NL = normal.

DISCUSSION

Physicians are faced with a significant challenge when treating life-threatening anemia in patients unwilling to accept blood for religious reasons or for whom safe blood is not available due to extensive alloimmunization or lack of supply in some developing countries.[3,6,15-19] Unfortunately, survival of patients with life-threatening anemia for whom RBC transfusion is not an option has not improved over the past 20 or 30 years.[20,21] Administration of HBOCs may represent a therapeutic alternative to reduce mortality for both medical and surgical patients in this population.[22] One option is HBOC-201, an acellular bovine Hb product. The biochemical properties of HBOC-201 offer potential physiologic benefits for severely anemic patients in SCC and have demonstrated safety and improved exercise tolerance in sickle cell patients when not in crisis.[23] At 1×10^{-8} the size of an RBC, with lesser viscosity, HBOC-201 may bypass vasoocclusions, delivering oxygen to downstream ischemic tissue that larger RBCs cannot reach. It may also decrease diffusion distances between native RBCs and vascular endothelium.[8,11,24] Finally, HBOC-201 has a lower affinity for oxygen than native Hb (Fig. 2), allowing for more efficient off-loading of oxygen to ischemic tissues.[24-26] A potential benefit of HBOC-201 might therefore be early tissue reoxygenation, which may prevent or mitigate the damage from acute chest syndrome, a common and serious pathology of SCC.[27]

Red blood cells are known to lose 2,3-bisphosphoglycerate (2,3-BPG) during storage, and an in vivo regeneration time of approximately 4 hours is required to restore the concentration to about 50% normal.[7,9,11,28,29] In the absence of adequate 2,3-BPG, the affinity of Hb for oxygen may be too high to optimally off-load oxygen to ischemic tissue.[1,28] In contrast, HBOC-201 can immediately carry and deliver oxygen as its affinity for oxygen is not influenced by 2,3-BPG. Thus, patients with clinically severe anemia may theoretically benefit more rapidly from HBOC-201 than blood that has been stored for an extended period. However, this view has been challenged by Weiskopf and colleagues,[30] who demonstrated stored blood to be equally efficacious to fresh blood in reversing cerebral ischemia, despite its lower P50.

Here, we document administration of HBOC-201 to three individuals in SCC who were not simply anemic but additionally had multisystem organ dysfunction. Blood was not an option for the two JWs, and compatible blood was not available for the other patient. All three patients developed acute chest syndrome with hypoxic respiratory failure and Hb level less than 4 g/dL. Two patients developed heart and liver failure, one of whom also developed renal failure. Two patients received more than 20 units of HBOC-201, while the other received only 6. The only adverse effect he experienced was transient methemoglobinemia, which, while not directly toxic, decreases the amount of physiologically useful Hb. The 27 units used in the third case equal the largest volume a patient has received to date and survived (Z. Zafirelis, CEO of HbO2 Therapeutics, personal communication, June 9, 2017). This is equal to the dose cited in a case by Epperla and coworkers.[31]

HBOC-201 has been administered to more than 800 anemic patients in 22 clinical trials and was evaluated as an alternative to blood transfusion in a multicenter elective orthopedic surgery Phase III trial.[32] This study demonstrated a 59% blood transfusion avoidance and no statistical difference in mortality between the HBOC-201 and RBC transfusion arms of the study. However, the number of serious adverse events was higher in the HBOC-201 arm (25% vs. 17%) including an increased risk of myocardial injury, stroke, and achalasia.[31] Due to the lack of mortality benefit and increased risk of adverse events, the FDA has only approved HBOC-201 for expanded access at this time. Nonetheless, preventing severe anemia is preferable to reversing it, and no studies to date have evaluated possible long-term neurologic deficits arising in patients who have survived a sustained period of severe anemia.[22]

PolyHeme (Northfield Laboratories) was another cell-free polymerized HBOC, which initially showed promise in a trial supporting trauma and surgical patients, including those requiring massive transfusions.[33] It was also used to support a JW patient during acute chest syndrome with acute respiratory failure due to *Staphylococcus* bacteremia and respiratory failure, and another JW patient with acute chest syndrome and respiratory failure due to *Staphylococcus aureus* bacteremia and pulmonary embolism; this product is no longer available.[13,34,35]

Two of the three patients in our case series developed methemoglobinemia, and two had transient hypertension.

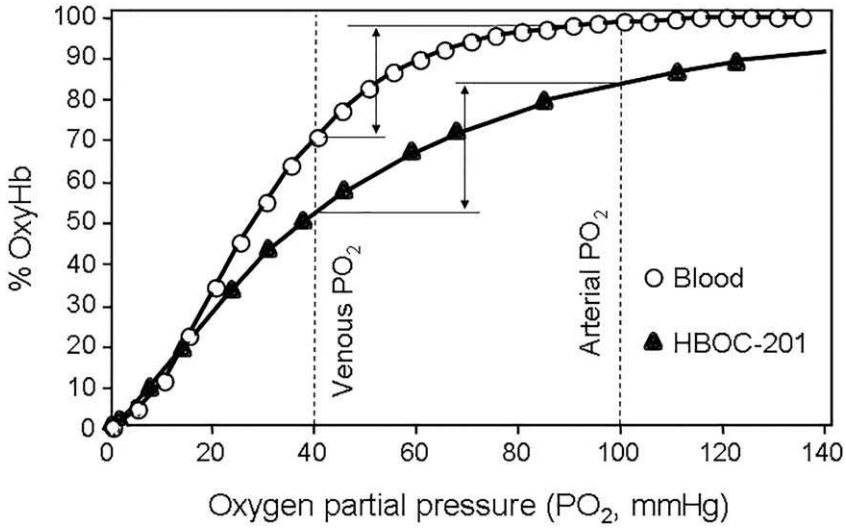

Fig. 2. Hb dissociation curves for Hemopure and RBCs. At an arterial PO_2 of 100 mmHg, corpuscular Hb is 98% saturated and Hemopure is 85% saturated. However, for a given decrease in PO_2 from 100 to 40 mmHg, as blood travels from the lungs to peripheral tissues, the fraction of total oxygen released by HBOC-201 is greater than or equal to that released by corpuscular Hb to target organs. Vertical arrows represent the percent oxygen extraction between arterial and venous PO_2 levels. Adapted and reprinted from EuroIntervention 4, Dubé et al, HBOC-201: The Multi-purpose Oxygen Therapeutic, p. 161-5, Copyright (2008), with permission from Europa Digital & Publishing.

The hypertension associated with HBOC-201 administration is posited to arise from free Hb scavenging nitric oxide, with resultant vasoconstriction.[9,36] The two patients with methemoglobinemia were treated with ascorbic acid, one patient orally and the other IV. One patient with hypertension was treated with a single dose of labetalol. Despite these minor adverse events, all three patients made full recoveries and were discharged after hospital stays greater than 1 month. None of the patients had neurologic dysfunction.

HBOC-201 can be obtained for expanded access by contacting Z. Zafirelis at +1 (781) 373-1848, and more information on the product is available at www.HbO2-Therapeutics.com. Institutional review board approval is required within 5 working days of use for expanded access products.

In conclusion, supportive measures were exhausted in all three patients before the initiation of HBOC-201. These critically ill patients with multiorgan failure experienced minimal adverse effects, all survived, and the two JWs did not violate their religious creed. These reports suggest that artificial oxygen carriers such as HBOC-201 can provide an oxygen bridge in SCC, until corpuscular Hb levels recover to meet metabolic demand, and highlight the compelling biochemical properties that warrant further investigation.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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