Bovine Blood and Neuromuscular Paralysis as a Bridge to Recovery in a Patient with Severe Autoimmune Hemolytic Anemia

Monvasi Pachinburavan, M.D. and Paul E. Marik, M.D., F.A.C.P., F.C.C.M.

Abstract

Hemoglobin solutions have several advantages as substitutes for erythrocyte transfusions. They have a prolonged shelf life, do not require cross-matching, are associated with few transfusion reactions, and are effective in delivering oxygen to the tissues. Despite the potential clinical utility of these solutions, they have not achieved widespread use. HbOC-201 (Hemopure, Biopure, Cambridge, MA, USA) is a bovine hemoglobin solution that has been FDA approved for compassionate use in Jehovah's witness patients requiring transfusions. Here we report a case of a patient with hemoglobin H disease who developed a severe life-threatening mixed warm and cold antibody mediated autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP) who was successfully treated with HbOC-201 as an adjunct to blood transfusion.

Keywords: autoimmune hemolytic anemia, immune thrombocytopenia, bovine hemoglobin, blood substitute, Hemopure, Evans syndrome, ICU, paralysis

Case Report

A 28-year-old Asian male with no known past medical history presented to our hospital with a 1-week history of shortness of breath, light-headedness, and fatigue. He had presented earlier to his primary care physician with several days of nonproductive cough and low-grade fever and was treated with Azithromycin. He denied any history of bleeding, trauma, or previous blood transfusions. His only other medication was ibuprofen. On physical examination, he was normotensive but tachycardiac and tachypneic with a heart rate of 120 beats/minute and respiratory rate of 26 per minute. There was no hepato-splenomegaly, ecchymoses, or petechia noted. His initial laboratory results were as follows: hemoglobin 1.7 g/dL with 0.1% reticulocytes, hematocrit (Hct) 6.2%, white blood cell count $6.7 \times 10^9/L$ with a normal differential count and platelet count of $17 \times 10^9/L$; the peripheral blood smear showed microspherocytes, polychromasia, and decreased platelets. The INR was 1.86, lactate 47 mg/dL (normal 2–20 mg/dL), total bilirubin 3.1 mg/dL, direct bilirubin 0.7 mg/dL, serum lactate dehydrogenase 534 U/L, haptoglobin <6 mg/dL, with normal serum chemistries. Direct Coomb's test was positive (for IgG and complement), with both warm and cold autoantibody reactivity. A presumptive diagnosis of AIHA with thrombocytopenia was made (Evans syndrome) and the patient was started on methylprednisolone 60 mg/day and empirical broad spectrum antibiotics.

Within 8 hours of admission, the patient developed worsening metabolic acidosis and respiratory distress. Arterial blood gas on 100% rebreathing mask showed a pH of 7.16, PaCO₂ 16 mmHg and PaO₂ 186 mmHg. His chest X-ray showed pulmonary congestion. A decision was made to intubate and paralyze the patient and place him on mechanical ventilation to decrease the work of breathing and to minimize metabolic activity. Rectal temperature was maintained at 37°C to minimize hemolysis secondary to cold autoantibodies. Norepinephrine was required for hemodynamic instability. The dose of methylprednisolone was increased to 1 g/day (for total of 3 days) and intravenous immune globulin was administered (5 g/kg for total of 5 days).

Cross-matched blood was ordered; however, due to the presence of allo- and autoantibodies, it was extremely difficult to find blood for the patient. Since the patient's condition was rapidly

deteriorating, informed consent from the family was obtained to administer bovine hemoglobin (HBOC-201) as an adjunctive measure until conventional blood transfusion was available and the AIHA had improved. Approval for compassionate use of bovine hemoglobin was also obtained from the hospital's institutional review board and the Food and Drug Administration (FDA). A single transfusion of two units of bovine hemoglobin was administered to the patient at a rate of 0.5 g/minute. With the institution of these measures, the patient's metabolic status improved (pH 7.31, PaCO₂ 28 mmHg, PaO₂ 368 mmHg, and lactate of 21.7 mg/dL). The patient received four units of "least incompatible" blood and platelet transfusions over the subsequent 48 hours. During this time, the lactate continued to fall (to 15.8 and 11.0 mg/dL) with correction of the metabolic acidosis. The FiO₂ was gradually weaned and the patient was then successfully extubated. The methemoglobin level had increased to 7.9%; this was treated with methylene blue and subsequently fell to 2.9%. Due to the very low reticulocyte count, a bone marrow aspiration and biopsy was performed. This demonstrated a markedly hypercellular bone marrow with megakaryocytic and erythroid hyperplasia with a maturation arrest in erythropoiesis and no evidence of an infiltrative process. Flow cytometry revealed no evidence of B- or T-cell monoclonality or aberrant antigen expression. Additional laboratory testing including measurement of heavy metals and serology for HIV, *Mycoplasma*, parvovirus B19, hepatitis A, B, and C, as well as autoantibodies, including antinuclear antibody (ANA), double-stranded DNA (dsDNA), rheumatoid factor, and cardiolipins, were all negative. Serum immunoglobulins and immunoglobulin subclasses and G6PD levels were normal. Hemoglobin electrophoresis was compatible with hemoglobin H disease. Over the course of the following 2 weeks, the patient's reticulocyte count gradually increased, reaching 37%, the platelet count increased to $68 \times 10^9/L$, and his hemoglobin stabilized at around 9 mg/dL (Figure 1). The patient's clinical condition improved markedly and he was subsequently discharged home.

Discussion

Evans syndrome is a rare disorder characterized by the combination of AIHA and ITP, in the absence of a known etiology.¹ Although

Division of Pulmonary and Critical Care Medicine, Thomas Jefferson University, 834 Walnut Street, Suite 650, Philadelphia, Pennsylvania, USA.

Correspondence: P Marik (paul.marik@jefferson.edu)

DOI: 10.1111/j.1752-8062.2008.00006.x

Figure 1. Patient's blood count profile over time.

Evans syndrome appears to be a disorder of immune regulation, the exact pathophysiology is unknown. The diagnosis is made by excluding other causes of acquired immune cytopenias, including SLE, IgA deficiency, acquired immunodeficiency syndrome, and autoimmune lymphoproliferative syndrome (ALPS).² ALPS is a disorder of defective lymphocyte apoptosis which usually presents in childhood. Measurement of peripheral blood T-cell subsets by flow cytometry allows differentiation of ALPS from Evans syndrome.² Our case is notable for the extreme anemia (a hemoglobin of 1.7 g/dL, which is not compatible with sustained life) and the profound and prolonged reticulocytopenia (0.1%). Severe reticulocytopenia associated with AIHA is uncommon and has been reported to be associated with immune mediated apoptosis of bone marrow erythroblasts. The bone marrow findings in our case would be compatible with this observation.

Two main interventions were predominant in the management of our patient with life-threatening AIHA. First is the use of mechanical ventilation and neuromuscular paralysis to decrease the work of breathing and minimize metabolic activity. Second was the use of polymerized bovine hemoglobin as an adjunctive measure to conventional blood transfusion to improve tissue oxygen delivery while awaiting the response to conventional treatment (corticosteroids and IVIG). We believe that this is the first use of such a therapeutic approach in the management of a patient with combined AIHA and ITP.

Hemoglobin-based oxygen carriers contain cell-free hemoglobin that have been modified to mimic human blood in oxygen carrying capacity. Hemoglobin solutions can be prepared from outdated human blood, bovine hemoglobin, or recombinant human hemoglobin. The hemoglobin is modified by polymerization and cross-linkage to maintain intravascular stability and prevent nephrotoxicity secondary to renal deposition of heme breakdown products. The nitric oxide scavenging effects of hemoglobin solutions have vasopressor effects. When stripped of the cell membranes, human hemoglobin carries low 2,3 diphosphoglycerate, causing high oxygen affinity, and poor tissue release. The affinity of bovine hemoglobin for oxygen is, however, regulated by serum chloride ions, resulting in excellent oxygen transport properties. Polymerized bovine hemoglobin is smaller, less viscous, and more readily releases oxygen than human red blood cells.

HBOC-201 is a purified, cell free, glutaraldehyde cross linked, polymerized bovine hemoglobin in a modified Ringer's solution with a circulatory half-life of 19 hours. It can be stored at room temperature for up to 3 years. Since there are no cell membranes, there is decreased antigenicity and cross-matching is not necessary. The properties of bovine hemoglobin solutions have been recognized as potentially beneficial in emergency and combat situations.⁴ Indeed, phase III trials using bovine hemoglobin in vascular, cardiac, and orthopedic surgery have shown to reduce utilization of conventional transfusion.⁵⁻⁷ The decreased antigenicity of hemoglobin solutions is a potentially useful option in patients with hemolytic disease. Mullon and colleagues have previously reported the successful use of HBOC-201 in a woman with severe AIHA (Hct 7.5%).⁸ There have been subsequent reports of the use of bovine hemoglobin in other hemoglobinopathies such as sickle cell disease.^{9,10}

There has been some concern that bovine hemoglobin solutions may be antigenic. This may be potentially problematic in patients with hemoglobinopathies who may require multiple transfusions. However, Hamilton and Kickler demonstrated that while IgG anti-HbOC-201 antibodies can be detected in the serum of human subjects after the transfusion of HbOC-201, it did not cause hemolysis of human red cells.¹¹

In summary, we present a patient with severe AIHA and ITP with reticulocytopenia who received bovine hemoglobin and neuromuscular paralysis as adjunctive therapy to conventional blood transfusions, IVIG, and a high dose methylprednisolone. The patient made an otherwise uneventful recovery.

Conflict of Interest

The authors have no financial interest in any of the products mentioned in this article.

References

- Norton A, Roberts I. Management of Evans syndrome. *Br J Haematol*. 2006; 132: 125–137.
- Teachey DT, Manno CS, Axsom KM, Andrews T, Choi JK, Greenbaum BH, McMann JM, Sullivan KE, Travis SF, Grupp SA. Unmasking Evans syndrome: T-cell phenotype and apoptotic response reveal autoimmune lymphoproliferative syndrome (ALPS). *Blood* 2005; 105: 2443–2448.
- Van De Loosdrecht AA, Hendriks DW, Blom NR, Smit JW, De Wolf JT, Vellenga E. Excessive apoptosis of bone marrow erythroblasts in a patient with autoimmune haemolytic anaemia with reticulocytopenia. *Br J Haematol*. 2000; 108: 313–315.
- Patel MB, Feinstein AJ, Saenz AD, Majetschak M, Proctor KG. Prehospital HBOC-201 after traumatic brain injury and hemorrhagic shock in swine. *J Trauma*. 2006; 61: 46–56.
- Levy JH, Goodnough LT, Greilich PE, Parr GV, Stewart RW, Gratz I, Wahr J, Williams J, Comunale ME, Doblar D, Silvey G, Cohen M, Jahr JS, Vlahakes GJ. 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.
- Sprung J, Kindscher JD, Wahr JA, Levy JH, Monk TG, Moritz MW, O'Hara PJ. The use of bovine hemoglobin glutamer-250 (Hemopure) in surgical patients: results of a multicenter, randomized, single-blinded trial. *Anesth Analg*. 2002; 94: 799–808.
- Jahr JS, Dulchavsky SA, Garino JP. Hemodynamic effects of hemoglobin glutamer-250 (Hemopure, HBOC-201) compared to red cells in pivotal safety and efficacy study in orthopedic surgery patients. *Anesth Analg*. 2002; 94: S330.
- Mullon J, Giacompe G, Clagett C, McCune D, Dillard T. Transfusion of polymerized bovine hemoglobin in a patient with severe autoimmune hemolytic anemia. *N Engl J Med*. 2000; 342: 1638–1643.
- Stefan DC, Uys R, Wessels G. Hemopure transfusion in a child with severe anemia. *Pediatr Hematol Oncol*. 2007; 24: 269–273.
- Gonzalez P, Hackney AC, Jones S, Strayhorn D, Hoffman EB, Hughes G, Jacobs EE, Orringer EP. A phase I/II study of polymerized bovine hemoglobin in adult patients with sickle cell disease not in crisis at the time of study. *J Invest Med*. 1997; 45: 258–264.
- Hamilton RG, Kickler TS. Bovine hemoglobin (glutamer-250, Hemopure)-specific immunoglobulin G antibody cross-reacts with human hemoglobin but does not lyse red blood cells *in vitro*. *Transfusion* 2007; 47: 723–728.