In vitro effects of a novel hemoglobin-based oxygen carrier on routine chemistry, therapeutic drug, coagulation, hematology, and blood bank assays

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Red blood cell (RBC) replacement solutions are being developed as alternatives to allogeneic RBC use in blood transfusions in the treatment of massive trauma, to achieve hemodynamic stability during elective surgery, and to increase oxygen-carrying capacity in anemia. Hemoglobin-based oxygen carrier (HBOC)-201 (Biopure Corp.) is a purified, sterile, isosmotic glutaraldehyde-polymerized bovine hemoglobin. Because this product is acellular, blood components containing this substance appear hemolyzed. This study reports on the interferences produced by the presence of HBOC-201 in a variety of clinical assays. This product was added in vitro at concentrations up to 60 g/L (6.0 g/dL) to normal human serum, plasma, or whole blood before testing for serum chemistries, coagulation profiles, and hematology and blood bank assays. In addition, a set of normal human sera containing HBOC-201 was supplemented with various therapeutic drugs and assayed for these agents. The results of these studies demonstrate that the presence of HBOC-201 in blood components does not result in significant analytical interference that would be of concern with many clinical assays encountered during routine clinical use of this RBC replacement solution in patients.

Population studies project doubling of the population over 65 years of age in the next 30 years, resulting in a projected annual deficit of 4 million units of blood by the year 2030 [3, 4]. Strategies to minimize blood use such as lowering the transfusion trigger to 80 g/L (8 g/dL) hemoglobin, using autologous transfusions, using recombinant erythropoietin, and achieving normovolemic hemodilution will not cover this projected deficit [2, 3]. Thus, in addition to secondary concerns such as undetected infectious agents in allogeneic blood transfusions, transfusion reactions, cost, and storage issues [5–7], the projected blood unit deficit in the coming years is a driving force for the development and utilization of blood substitutes. Blood substitutes can be defined as oxygen-carrying volume expanders [3]. An optimal product should have oxygen delivery capabilities at physiologic oxygen partial pressures, be readily available, and be competitive with human blood in terms of cost as well as safety (infection free and nonantigenic) [5–7].

Hemoglobin-based oxygen carrier (HBOC)-201 is an investigational solution of polymerized bovine hemoglobin manufactured by Biopure Corp., with a plasma half-life of 16–24 h.2 Because of its acellular nature, the presence of this product in blood components imparts a hemolytic appearance to specimens containing this product. In turn, this can potentially complicate various laboratory assays that are routinely used for the monitoring of patients receiving this product. Selected aspects concerning hemolytic interference resulting from the presence of HBOC-201 in serum, plasma,
Materials and Methods
HBOC-201 is a purified, sterile, isosmotic glutaraldehydepolymerized bovine hemoglobin manufactured by Biopure Corp. This product has concentrations of total hemoglobin 120–140 g/L (12–14 g/dL), sodium 145–160 mmol/L, chloride 105–120 mmol/L, potassium 3.5–5.5 mmol/L, calcium 0.5–1.5 mmol/L, pH 7.6–7.9, and osmolality 290–310 mosmol/kg. The absorption spectrum of serum containing HBOC-201 was recorded with a PerkinElmer Corp. Lambda 3 UV/VIS spectrophotometer (Coleman Instruments Div.). HBOC-201 was diluted in normal human serum to various concentrations ranging from 0.50–50 g/L (0.05–5.0 g/dL) (the range of HBOC-201 expected to be encountered in specimens from patients treated with this product). Each sample was frozen at −20 °C and thawed before testing. With the Paramax RX analyzer (Dade International) and manufacturer-specified reagents, the HBOC-201 serum specimens were assayed for several analytes, including sodium, chloride, potassium, glucose, creatine kinase (CK), blood urea nitrogen (BUN), cholesterol, calcium, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), creatinine, alkaline phosphatase (ALP), amylase, magnesium, phosphorus in the form of phosphate ions, total bilirubin, and direct bilirubin. The same HBOC-201 serum samples were analyzed on the Synchrom CX3 analyzer (Beckman Instruments) with the manufacturer-specified reagent kit for CK-MB. Lipase concentrations in the same samples were also assessed on the Stratus II analyzer (Dade International) with the manufacturer-specified reagent kit for CK-MB. Lipase concentrations in the same samples were also assayed on the BMC/Hitachi 717 automated analyzer (Boehringer Mannheim Corp.) with the appropriate manufacturer-specified reagents for lipase. Reconstitution of HBOC-201 in freshly drawn normal whole blood was carried out at concentrations up to 65 g/L (6.5 g/dL) for analysis with the Chiron Diagnostic Corp. 865 cooximeter (East Walpole, MA) for hemoglobin, chloride, potassium, sodium, glucose, and ionized calcium. The same HBOC-201 whole-blood samples were also evaluated on the ICA2 Radiometer for ionized calcium concentrations. A set of serum samples containing various concentrations of HBOC-201 and fixed concentrations of Dilantin (phenytoin), gentamicin, lidocaine, N-acetylcysteamine, procainamide, quinidine, theophylline, and vancomycin were prepared and refrigerated at 4 °C. These samples were analyzed for these therapeutic drugs on the TDxFLx analyzer (Abbott Labs.) with the manufacturer-specified reagent packs. A set of plasma samples containing various concentrations of HBOC-201 and saline was prepared and frozen at −20 °C. These samples were thawed before determining the fibrinogen concentrations, activated partial thromboplastin time (aPTT), and prothrombin time (PT) with BBL® Fibrosystem® fibrometers (Becton Dickinson) and Dade International reagents for these tests. A set of HBOC-201 specimens was reconstituted with normal freshly drawn whole blood to various concentrations of HBOC-201 up to 65 g/L (6.5 g/dL). These samples were analyzed for components in the complete blood count (CBC) and white blood cell (WBC) differential with the Cell-Dyn 3500 (Abbott Labs.). These samples were also analyzed for reticulocytes on the Sysmex™ Model R-3000™ reticulocyte analyzer (TOA Medical Electronics). HBOC-21 was added at different concentrations to normal human whole blood (type A positive and type B positive) and cross-matched. Unless the patient has an alloantibody, testing for other minor blood group antigens is not performed. These samples were treated as normal patient samples to detect ABO incompatibility. HBOC-21 was added to incompatible mixed blood (type O positive and type A positive) at the same concentrations (up to 65 g/L) and analyzed with the Gamma-clone® antiglobulin test (Gamma Biologicals). Immediate spin phase of the cross-match tests for IgM isoagglutinins and the 37 °C and Coombs’ phase tests for IgA antibodies. No antibodies related to ABO blood group antigens were detected.

All analytical instrument operators were blinded to analyte and HBOC-201 concentrations in the samples tested. The results were returned to Biopure Corp. where they were analyzed and the maximum concentrations of HBOC-201 that did not produce interference in each assay were collaboratively determined.

Results
The light absorption spectrum of a serum specimen containing HBOC-201 is shown in Fig. 1. The presence of HBOC-201 resulted in three peaks at 415, 540, and 576 nm, which are characteristic of oxyhemoglobin.

The results from the interference studies regarding the presence of HBOC-201 in serum tested for various analytes on the Paramax RX instrument are presented in Fig. 2. HBOC-201 did not result in analytical interference at concentrations up to 50 g/L (5.0 g/dL) in the sodium, chloride, and potassium assays—all ion-selective electrode (ISE) methods. The remaining assays investigated on the Paramax RX are colorimetric methods. HBOC-201 concentrations ≤45 g/L (4.5 g/dL) did not interfere with the glucose and CK measurements. However, at concentrations exceeding 45 g/L, HBOC-201 produced negative interference (amount of analyte measured was lower than what was present). BUN and cholesterol assays were not...
affected by HBOC-201 concentrations up to 40 g/L (4.0 g/dL), whereas at higher concentrations a positive interference in the cholesterol assay were noted. Calcium, total protein, ALT, AST, GGT, and LDH measurements were unaffected by the presence of HBOC-201 up to 25, 12, 10, 8.0, 6.0, and 6.0 g/L (2.5, 1.2, 1.0, 0.8, 0.6, and 0.6 g/dL) respectively, whereas at higher concentrations HBOC-201 produced positive interferences in these assays. Albumin and creatinine measurements were unaffected by the presence of HBOC-201 up to 7.0 and 4.0 g/L (0.7 and 0.4 g/dL) respectively, with exceeding concentrations producing negative interferences. Filtration of sera before measurement of creatinine removed all interferences at the highest HBOC-201 concentrations tested (HBOC-201 concentrations before filtration were up to 65 g/L). ALP, amylase, direct bilirubin, total bilirubin, magnesium, and phosphorus concentrations could not be assessed in the visible presence of HBOC-201 (amount of hemolysis of 1.0 g/L and graded as +1 on a scale from 1 to 4) with the chromogenic methods on the Paramax RX analyzer. Specifically, HBOC-201 produced a large negative interference in the measurement of ALP and phosphorus, and a large positive interference in amylase, magnesium, and total and direct bilirubin quantification.

The same set of HBOC-201-supplemented sera was also studied with the Synchron CX3 analyzer by testing for various analytes. The results of these studies are depicted in Fig. 3. Sodium, potassium, and chloride were measured by ISE methods and were unaffected by HBOC-201 concentrations up to 50 g/L (5.0 g/dL). BUN determinations based on an enzymatic conductivity rate method were also unaffected by HBOC-201 concentrations up to 50 g/L. Glucose was measured by an oxygen consumption rate method and was unaffected by the presence of HBOC-201 at 50 g/L. On the other hand, measurement of creatinine with a colorimetric method was not possible in the visible presence of HBOC-201, which produced a substantial negative interference. However, filtration of these sera before testing for creatinine removed all interfering substances.

The results of other miscellaneous studies generally demonstrated minimal HBOC-201 interference. CK-MB concentrations were determined on the Stratus II analyzer with a fluorometric enzyme immunoassay. CK-MB measurement was not affected by the presence of HBOC-201 at maximum concentrations (50 g/L). HBOC-201 up to 45 g/L (4.5 g/dL) did not affect lipase determinations on the Hitachi 717 analyzer with a turbidimetric method. At higher HBOC-201 concentrations, a negative interference in lipase measurements was observed.

Normal human whole blood supplemented with various concentrations of HBOC-201 was analyzed on the Chiron 865 analyzer for chloride, potassium, sodium, and ionized calcium with ISE technology; for glucose with an
amperometric method; and for hemoglobin with a spectrophotometric method. The maximum HBOC-201 concentration that produced no interference in these assays is depicted in Fig. 4. With the exception of glucose, none of these assays was affected by HBOC-201 concentrations up to 65 g/L (6.5 g/dL). Glucose measurement demonstrated negative interference in the presence of HBOC-201 at concentrations >39 g/L (3.9 g/dL). The same HBOC-201 whole-blood samples were also evaluated on the ICA2 Radiometer for ionized calcium concentrations by ISE technology. HBOC-201 did not affect this measurement at concentrations as high as 65 g/L.

The same set of HBOC-201-supplemented serum samples containing various concentrations of HBOC-201 and fixed concentrations of various therapeutic drugs were analyzed for these drugs on the TDxFLx analyzer by fluorescence polarization immunoassays (FPIA). The results of these studies are presented in Fig. 5. With the exception of gentamicin and vancomycin, all other drug measurements were unaffected by HBOC-201 concentrations up to 50 g/L. Visible presence of HBOC-201 produced a positive interference in the measurement of gentamicin and a negative interference in the measurement of vancomycin on the TDxFLx.

Plasma samples containing various concentrations of HBOC-201 and saline were tested for fibrinogen concentrations, aPTT, and PT by using fibrometers with Dade reagents. These tests were unaffected by the presence of HBOC-201 up to 65 g/L. A set of HBOC-201 specimens was reconstituted with normal freshly drawn whole blood to various concentrations of HBOC-201. These samples were analyzed for CBC variables by electrical impedance technology and WBC differential variables by flow cytometry with the Cell-Dyn 3500. No interference by HBOC-201 up to 65 g/L was observed in any of the following measured or calculated variables with the Cell-Dyn 3500 tests for CBC and WBC differentials: hematocrit, red blood cell count, platelets, WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, mean corpuscular volume, mean platelet volume, and red cell distribution width. These samples were also analyzed for reticulocytes on the Sysmex R-3000 flow cytometer with no interference by the HBOC-201 product at maximum concentrations. HBOC-201 was added to normal human whole blood as well as to incompatible mixed blood and analyzed with an antiglobulin test, with no interference by HBOC-201 up to 65 g/L.

Discussion

One driving force for development of blood substitutes is the increasing need for supply of blood, exacerbated by decreasing frequencies of blood collection and an increasing frequency of surgical procedures in an aging population [4]. Although the potential complications associated with the administration and use of artificial blood products are multiple and have been investigated in depth [8], issues relating to the laboratory monitoring of patients receiving such products have not been published. In our reported studies the issue of interference by a HBOC in many routine laboratory procedures was investigated. There was minimal interference due to the presence of HBOC-201 in noncolorimetric assays. In general, the presence of HBOC-201 in various blood components does not interfere with the measurement of most analytes at expected in vivo HBOC-201 concentrations of 35 g/L (3.5 g/dL). Filtration of serum containing HBOC-201 before creatinine measurement removed interfering substances without affecting the true creatinine value, thus providing a practical and feasible solution to the monitoring of this critical analyte.

HBOC-201 added to sera did produce significant interference in assays of the following analytes: calcium, total protein, ALT, AST, albumin, GGT, LDH, ALP, amylase, magnesium, phosphorus, direct and total bilirubin, gentamicin, and vancomycin. These assays, with the exception of the therapeutic drugs, are photometric methods.
The analytical systems used in these studies monitor reaction rates bichromatically and detect hemolysis as well as other types of spectral interferences. But these systems do not appear to be designed to correct for higher concentrations of hemoglobin in hemolytic samples, comparable with the degree of spectral interferences represented by HBOC-201 in moderate to high concentrations. With the advent of newer technologies in which colorimetric reaction rates can be monitored at multiple wavelengths, instrument correction for any concentration of free hemoglobin present in a sample may become more widely used. Alternatively, filtration of samples containing HBOC-201, as performed with creatinine measurements, may resolve a degree of interference observed in existing methods, although this is time consuming. Another alternative rests on future development of nonphotometric methods for measurement of many routine analytes.

The interference noted with the monitoring of gentamicin and vancomycin is surprising, since these assays are based on the same methodology as other therapeutic drug monitoring assays (FPIA) conducted on the same analytical system. This suggests that HBOC-201 interference may be due to unique and complex interactions with some component(s) of this product. It may be that the interferent component is different in each of these two assays. The coagulation and hematology profiling of samples containing HBOC-201 was also not affected. This would be expected, since none of the methods used is based on spectrophotometric measurements.

The amount of hemoglobin present in any blood specimen (whole blood, serum, plasma) can be and has been quantified in our laboratories with the HemoCue® Blood Hemoglobin System. This photometric system is calibrated against a hemoglobin cyanide method for the determination of total hemoglobin concentration in blood. The lower limit of sensitivity of this system is 1.0 g/L (0.1 g/dL), which corresponds to a visible amount of hemolysis graded as +1.

In conclusion, use of HBOC-201 does not interfere with many clinical laboratory tests to the extent of negating its clinical use as an artificial HBOC.

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References