The Use of Bovine Hemoglobin Glutamer-250 (Hemopure®) in Surgical Patients: Results of a Multicenter, Randomized, Single-Blinded Trial

Juraj Sprung, MD, PhD*, James D. Kindscher, MD‡, Joyce A. Wahr, MD§, Jerrold H. Levy, MD‖, Terri G. Monk, MD¶, Mark W. Moritz, MD#, and Patrick J. O'Hara, MD†

Departments of *Anesthesiology and †Vascular Surgery, The Cleveland Clinic Foundation, Cleveland, Ohio; ‡Department of Anesthesiology, University of Kansas Medical Center, Kansas City, Kansas; §Department of Anesthesiology, University of Michigan Medical Center, Ann Arbor, Michigan; ‖Department of Anesthesiology, Emory University Hospital, Atlanta, Georgia; ¶Department of Anesthesiology, Washington University School of Medicine, St. Louis, Missouri; and #Department of Surgery, Morristown Memorial Hospital, Morristown, and University of Medicine and Dentistry of New Jersey, Newark, New Jersey

Hemoglobin-based oxygen carrier-201 (HBOC-201, hemoglobin glutamer-250 [bovine], Hemopure®; Biopure Corporation, Cambridge, MA) is polymerized hemoglobin of bovine origin being developed as an oxygen therapeutic. In this study, we evaluated the tolerability of a single intraoperative dose of HBOC-201 in surgical patients. In a single-blinded, multicenter study, 81 patients were randomized to receive either a single infusion of HBOC-201 (55 patients) or an equivalent volume of lactated Ringer's solution (26 patients). Forty-two patients originally assigned to the HBOC-201 group received the entire planned treatment of only one of the following doses: 0.6, 0.9, 1.2, 1.5, 2.0, or 2.5 g/kg of body weight. Thirteen of the 55 patients in the HBOC-201-assigned group did not reach the trigger point for transfusion administration, and they were not included in the analysis. We studied clinical outcomes and compared hematologic findings, blood chemistry values, and blood use in the two treatment groups. There were no patient deaths in this study. No pattern of clinically significant laboratory abnormalities could be attributed to exposure to HBOC-201. In the HBOC-201 group, 2 patients had a transient increased concentration of serum transaminases and 6 had transient skin discoloration. One patient in the HBOC-201 group had mast cell degranulation with hypotension. Postoperatively, methemoglobin plasma concentrations increased in the HBOC-201 group in a dose-dependent manner, reaching maximal values of 3.7% ± 3.2% (average of all doses given) on postoperative day 3. There was no difference in the mean number of allogeneic blood units transfused in the 2 groups (3.3 ± 1.8 and 3.7 ± 4.1 for the lactated Ringer's solution and HBOC-201 groups, respectively) over the course of hospitalization. The intraoperative administration of HBOC-201, up to a maximum of 245 g, was generally well tolerated. There was no relationship between HBOC-201 use and the number of allogeneic blood units transfused over the entire hospitalization course. The administration of HBOC-201 was associated with a delayed (third postoperative day) dose-dependent increase in the plasma methemoglobin concentration. We conclude that the intraoperative use of HBOC-201 was generally well tolerated.

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Limitations of the blood supply and deficiencies in blood storage technology, together with adverse clinical experiences related to blood transfusions, have created an interest in the development of oxygen-carrying solutions to replace blood transfusions (1). Among the shortcomings currently associated with the collection, storage, and transfusion of blood are 1) the loss of 2,3-diphosphoglycerate in stored erythrocytes, causing reduction of oxygen release to tissues (2,3); 2) a human transfusion-related error (4); 3) the transmission of potentially fatal viral, bacterial, and parasitic infections (5–8); 4) immunomodulation (6); and 5) lack of blood for persons with particular medical conditions or alternatives to blood for patients whose religious or other beliefs prohibit blood transfusion (6,7,9,10). A replacement solution

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TGM is presently affiliated with the University of Florida College of Medicine, Gainesville, FL.

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Address correspondence and reprint requests to Juraj Sprung, MD, PhD, Department of Anesthesiology, Mayo Clinic, 200 First St. SW, Rochester, MN 55905.

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for blood transfusion would alleviate these problems. An ideal blood substitute would need to efficiently transport oxygen, dissociate appropriately from oxygen at the tissue level, be nontoxic and free of pathogens, and be available in adequate supplies at an acceptable cost.

Hemoglobin glutamer-250 (bovine) (hemoglobin-based oxygen carrier-201 [HBOC-201], Hemopure®; Biopure Corporation, Cambridge, MA) is an ultrapurified, glutaraldehyde-polymerized, bovine hemoglobin in a balanced electrolyte solution. The bovine hemoglobin in HBOC-201 has a molecular structure similar to that of human hemoglobin. The oxygen affinity is independent of 2,3-diphosphoglycerate, is not affected by storage, and is regulated by chloride ion (11,12). The P_{50} of HBOC-201 is between 36 and 38 mm Hg. Finally, HBOC-201 transports oxygen in the plasma phase of blood more efficiently than intracellular hemoglobin in erythrocytes, on the basis of enhanced oxygen on-loading and off-loading (13). These properties suggest a great potential of HBOC-201 as an oxygen carrier in surgical procedures that conventionally require blood transfusion.

In the present randomized, placebo-controlled trial, we evaluated the tolerability of HBOC-201 administered to surgical patients.

Materials and Methods

This was a multicenter (6 sites), randomized, single-blinded, placebo-controlled study of the tolerability of HBOC-201. The control group received a volume of lactated Ringer's (LR) solution equivalent to the volume of HBOC-201 given at each specified dose (see below). Before the intraoperative administration of clinical trial material, each patient randomized to the specific treatment group received a 5-mL test dose of either HBOC-201 or LR within 1 h before anesthesia. After a 15-min observation period and confirmation that the test dose was well tolerated, the patients were eligible to receive only one of the assigned infusions. The trigger for infusion was an estimated blood loss of at least 500 mL, with an additional surgical blood loss expected to exceed 500 mL. HBOC-201 or LR was administered by IV infusion via a dedicated line. The administered single doses of HBOC-201 were 0.6, 0.9, 1.2, 1.5, 2.0, and 2.5 g/kg of body weight. Each dose was administered to only one group of patients (Table 1 shows the number of patients and the total quantities of HBOC-201 solution administered to individual patient subgroups). At each dose, all patients were examined, and after no significant adverse effects were seen, the study was continued at the next-larger dose level (on different patients). The rate of administration of HBOC-201 and LR was 1.0 g/min (7.6 mL/min) for doses of 0.6, 0.9, and 1.2 g/kg and up to 3 g/min (22.8 mL/min) for doses of 1.5, 2.0, and 2.5 g/kg. For all patients in this study, all blood transfusion decisions for either allogeneic or autologous blood were exclusively based on the medical guidelines of the institution and clinician at each study site (e.g., hematocrit, total blood hemoglobin, and hemodynamic stability).

Table 1. Volumes of Infusions in the LR Solution Group and HBOC-201 Group (Overall and Subgroups)

	No. of Patients	Volume (mL)[a]
LR solution	26	849 ± 598
HBOC-201 subgroups (g/kg)		
0.6	7	380 ± 87
0.9	6	572 ± 155
1.2	11	755 ± 166
1.5	6	971 ± 213
2.0	6	1374 ± 182
2.5	6	1384 ± 309
HBOC-201 overall	42	875 ± 408

LR = lactated Ringer's, HBOC = hemoglobin-based oxygen carrier.
[a] Data are mean ± SD.

The Institutional Human Investigational Review Board at each site reviewed and approved the study protocol. After obtaining individual informed consent, 81 patients were enrolled in this clinical trial. These patients were scheduled for elective general or gastrointestinal, gynecologic, orthopedic, urologic, plastic reconstructive, and major vascular operations. Patients included in the study were men or women of nonchild-bearing potential, between 18 and 75 yr of age. Patients with severe cardiovascular disease (uncontrolled hypertension, unstable angina, myocardial infarction within the previous 6 mo, current congestive heart failure, or clinically significant peripheral vascular disease), severe pulmonary disease, uncontrolled endocrinologic disorder or hematologic disorder, biochemical or serologic evidence or history of acute or chronic hepatic disease or renal disease, or active infection or sepsis were all excluded from enrollment in the study. Patients with a history of allergic reactions to beef products were also excluded.

Of the 81 patients enrolled in the study, 26 were randomly assigned to the LR group and 55 to the HBOC-201 group (the study protocol was to enroll the patients in an approximately 2:1 ratio between HBOC-201 and LR). All patients in the HBOC-201 group received the 5-mL test dose. However, 1 patient had an adverse reaction to the test dose and 12 patients had insufficient intraoperative blood loss; therefore, they were excluded from further analysis, and 42 patients received the entire planned single-dose infusion of HBOC-201. One patient was reported previously (14).

Measurements and observations were made at 5 time points: 1) screening was done before or immediately after hospital admission, 2) the baseline period

Table 2. Demographic and Baseline Characteristics in Two Trial Groups

Characteristic	LR (n = 26)	HBOC-201 (n = 42)	P Value (between groups)
Men/women (n)	18:8	25:17	0.45[a]
Caucasian/African American (n)	26:0	37:5	0.15[a]
Age (yr)[b]	61.5 ± 9.7	61.3 ± 11.1	0.94
Height (cm)[b]	172.4 ± 10.4	170.1 ± 13.1	0.45
Weight (kg)[b]	83.3 ± 15.0	81.3 ± 17.5	0.62
Estimated blood loss (L)[b]	1.6 ± 1.1	1.7 ± 1.3	0.88
Duration of surgery (h)[b]	4.6 ± 2.1	5.1 ± 2.3	0.39

LR = lactated Ringer's solution, HBOC-201 = hemoglobin-based oxygen carrier-201.
[a] Fisher's exact test.
[b] Mean ± SD.

extended from screening until immediately before administration of the test dose of HBOC-201 or LR, 3) the perioperative treatment period began with the administration of the test dose and continued until 24 h after the start of the infusion of HBOC-201 or LR, 4) the postinfusion period extended from 24 h after the start of infusion through Day 7 or until hospital discharge, whichever occurred first, and 5) outpatient follow-up took place approximately 3 to 4 wk after surgery.

All adverse events were recorded. Each occurrence was analyzed with respect to association with clinical trial material. Throughout the study, we recorded the number of blood units given (autologous and allogeneic); total and plasma-free hemoglobin concentrations; methemoglobin concentrations; hematocrit (direct, spun method); reticulocyte count; erythropoietin and plasma iron levels; hemodynamic variables (blood pressure, heart rate); platelet count; fibrinogen levels; activated partial thromboplastin time, prothrombin time, alanine aminotransferase, aspartate aminotransferase, lipase, bilirubin (total, direct, and indirect), creatinine, blood urea nitrogen, and alkaline phosphatase values; serum electrolytes and glucose concentrations; and urinalysis findings. The length of hospitalization was calculated from the end of surgery to the day of discharge from the hospital.

Fifty-nine patients in this study were evaluated for immunoglobulin (Ig)G and IgE antibodies to HBOC-201 (IgG-aHBOC-201 and IgE-aHBOC-201, respectively); 19 were in the LR group and 40 in the HBOC-201 group. For each patient, a serum sample was collected at baseline and a second sample at least 2 wk after infusion of either LR or HBOC-201.

All of the randomized patients who received the full dose of HBOC-201 and all of the patients in the LR group were included in the analyses. The Mann-Whitney test or unpaired two-tail t-test was used for comparisons between the two means, as appropriate, and repeated analysis of variance with the Bonferroni test was used for multiple comparisons. The Fisher's exact test was used to test for differences in the distribution of categorical outcomes between groups. Values were expressed as means plus or minus standard deviation (±SD) or as medians and range, as appropriate. Statistical tests were two-sided, with the null hypothesis rejected at $P \leq 0.05$.

Results

There were no statistically significant differences in demographics between the two experimental groups (Table 2).

Serious Adverse Events

No patients died during the study. In the HBOC-201 group, 3 serious perioperative complications occurred. One patient with a history of mastocytosis had a histamine release reaction (see below) after receiving morphine followed by a test dose of HBOC-201. Two patients in the HBOC-201 group had cardiac complications postoperatively (congestive heart failure and myocardial infarction)—and both recovered. These two complications and several others that occurred in both treatment groups (diarrhea, small bowel obstruction, sepsis, infection) were not considered (by an unblinded investigator) to be related to either HBOC-201 or LR because they could have occurred as a result of the surgical procedures alone or underlying disease.

Minor Dermatologic Adverse Events

Six patients (11%) in the HBOC-201 group had dermatologic manifestations: 4 patients had skin (whole body) yellow discoloration (jaundice without an increase in serum bilirubin concentrations), and 2 had ecchymotic rash involving the areas underlying removed adhesives and sites of preoperative shaving. All dermatologic manifestations occurred in patients who received HBOC-201 in a dose of either 2.0 or 2.5 g/kg. No dermatologic adverse events occurred in the LR group.

Antibody Analysis

In the LR group, no patients were positive for IgG-aHBOC-201 or IgE-aHBOC-201 at baseline, and 2 patients (10.5%) had equivocal IgG-aHBOC-201 findings at follow-up (slight signal at the limit of detection of the assay). One patient with a history of mastocytosis was entered into the study. Just before the administration of the HBOC-201 test dose, the patient was given morphine. This patient then experienced immediate hypotension and diaphoresis, which resolved with fluids, ephedrine, phenylephrine, and diphenhydramine. After the event, the patient's serum tryptase concentration was 6.3 ng/mL (normal, <1.0 ng/mL), a confirmation of mast cell degranulation.

The patients in the HBOC-201 group who received the full HBOC-201 treatment were either negative (38 patients) or slightly positive (2 patients) for IgG-aHBOC-201 at baseline, and all patients were negative at both baseline and follow-up for IgE-aHBOC-201. At follow-up, 23 patients in this group (57.5%) had specific IgG-aHBOC-201 responses that were not associated with rash, itching, or flushing. Of these patients, 4 (17%) had maximal antibody concentrations of ≤1.5 kilo arbitrary units (kAU)/mL, which is close to the limit of detection of the assay; 12 had peak titers between 1.2 and 10 kAU/mL; and 7 had IgG-aHBOC-201 concentrations of >10 kAU/mL. The highest measured IgG-aHBOC-201 titer was 92 kAU/mL, and no hypersensitivity manifestations were recorded in this patient.

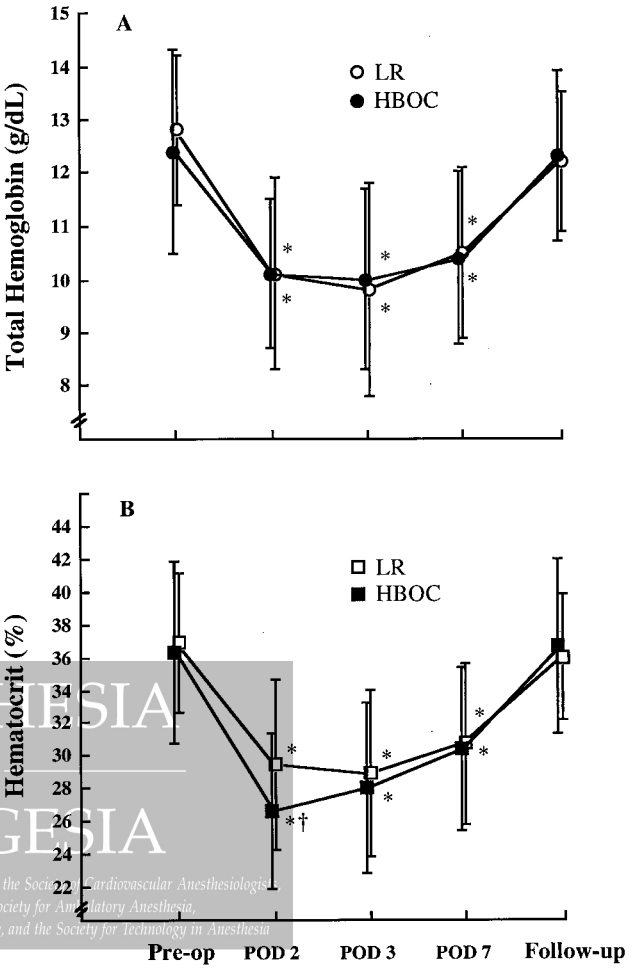

Figure 1. Changes in total hemoglobin concentration (A) and hematocrit (B) in patients who received either lactated Ringer's (LR) or hemoglobin-based oxygen carrier (HBOC)-201 solution. *$P < 0.001$ versus preoperative baseline; †$P < 0.03$ between the two groups, same time interval. POD, postoperative day; pre-op, preoperative. Data represent means and standard deviations for all patients in the study groups, irrespective of the amount of study solution infused.

Hemoglobin and Other Blood-Related Measurements

Total hemoglobin concentration comprises the red blood cell hemoglobin content and the plasma hemoglobin, which in this study reflects the presence of HBOC-201. In both groups, hemoglobin concentrations decreased postoperatively and returned toward baseline values by the day of outpatient follow-up. There was no difference between the two groups in the total hemoglobin concentrations measured at any time (Fig. 1A).

Mean hematocrit values were similar in the two groups before surgery, decreased postoperatively, and remained less than baseline values by postoperative day (POD) 7 ($P = 0.001$). Only on POD 2 was the hematocrit less in the HBOC-201 group (26.6%) than in the LR group (29.4%; $P = 0.03$) (information for more immediate postoperative times was not systematically obtained). At outpatient follow-up, hematocrits did not differ from group baseline values (Fig. 1B).

As expected, plasma hemoglobin levels were negligible in patients in both groups at baseline and at all measured times for patients in the LR group (Table 3). In the HBOC-201 group, plasma hemoglobin levels increased in a dose-dependent manner after the administration of HBOC-201 and decreased postoperatively but were still more than baseline values at POD 2 and POD 3 (both, $P < 0.001$) (Fig. 2). By POD 7 or discharge, plasma hemoglobin concentrations in the HBOC-201 subgroups were at baseline levels in the patients who received HBOC-201 in a dose ≤1.5 g/kg (baseline versus POD 7, $P > 0.67$) and more than baseline in those who received ≥2.0 g/kg ($P < 0.001$). Postoperatively, the percent of plasma methemoglobin increased in the HBOC-201 group and remained low in the patients who received LR. Although the percent of methemoglobin increased only slightly immediately after HBOC-201 infusion, the percent continued to increase during the postoperative period and peaked at POD 3 (mean maximum for all HBOC-201 patients was 3.7%) (Table 3). Figure 2 shows

Table 3. Plasma Hemoglobin, Methemoglobin, and Variables Related to Erythropoiesis

Variable	LR		HBOC-201		*P* Value (between groups)
	No. of Patients	Mean ± SD	No. of Patients	Mean ± SD	
Plasma hemoglobin (g/dL)					
Preoperative baseline	23	0.02 ± 0.04	31	0.03 ± 0.05	0.40
Immediately after infusion	21	0.02 ± 0.04	32	2.91 ± 1.07*	0.001
POD 1	22	0.03 ± 0.05	30	2.43 ± 1.05*	0.001
POD 2	21	0.01 ± 0.04	33	1.45 ± 0.97*	0.001
POD 3	22	0.02 ± 0.04	29	0.64 ± 0.61*	0.001
POD 7 or discharge	18	0.02 ± 0.04	30	0.16 ± 0.38	0.12
Methemoglobin (%)					
Preoperative baseline	24	0.58 ± 0.36	30	0.49 ± 0.26	0.30
Immediately after infusion	22	0.93 ± 0.46*	34	1.26 ± 0.49*	0.001
POD 1	23	0.93 ± 0.41*	32	1.62 ± 0.50*	0.001
POD 2	22	0.79 ± 0.31†	33	3.42 ± 1.66*	0.001
POD 3	22	0.73 ± 0.33	27	3.66 ± 3.18*	0.001
POD 7 or discharge	19	0.71 ± 0.42	26	1.13 ± 0.85‡	0.35
Reticulocyte count (%)					
Preoperative baseline	21	1.8 ± 0.9	32	2.1 ± 1.8	0.48
POD 3	20	1.8 ± 0.8	34	2.6 ± 1.9	0.08
POD 7 or discharge	21	2.4 ± 1.2	34	3.5 ± 2.0*	0.03
Outpatient follow-up	20	2.0 ± 1.5	32	1.9 ± 2.1	0.80
Erythropoietin (mU/mL)					
Preoperative baseline	25	14.4 ± 12.9	40	13.8 ± 9.3	0.82
POD 2	22	48.3 ± 41.9*	41	129 ± 279†	0.18
POD 3	22	62.9 ± 43.1*	37	67.4 ± 40.4*	0.68
POD 7 or discharge	18	49.4 ± 34.4*	36	45.5 ± 34.6*	0.70
Iron, total (mg/L)					
Preoperative baseline	10	0.9 ± 0.5	19	1.3 ± 2.1	0.60
POD 2	12	0.3 ± 0.2‡	19	43.2 ± 20.7*	<0.0001
POD 3	12	0.3 ± 0.2†	20	23.4 ± 15.3*	<0.0001
Outpatient follow-up	10	0.4 ± 0.2	17	5.1 ± 7.3	0.06

All values are averages for all HBOC-201 treatment subgroups.
LR = lactated Ringer's solution, HBOC-201 = hemoglobin-based oxygen carrier-201, POD = postoperative day.
Comparisons within treatments: * $P < 0.001$ versus preoperative baseline; † $P < 0.05$ versus preoperative baseline; ‡ $P < 0.01$ versus preoperative baseline.

plasma hemoglobin (panel A) and methemoglobin (panel B) in 6 HBOC-201 treatment subgroups. It is evident that methemoglobin concentrations were larger in subgroups who received larger doses of HBOC-201.

The reticulocyte count was not changed from the preoperative baseline value at any measured time in the LR group and was increased on POD 7 in the HBOC-201 group compared with the preoperative baseline value ($P = 0.001$) and with the LR value at POD 7 ($P < 0.03$) (Table 3). However, in both the LR and the HBOC-201 groups, erythropoietin concentrations increased at all measured postoperative periods. There were no differences in erythropoietin concentrations between the two groups at any measured intervals. During the first 3 PODs, total iron plasma concentrations decreased in the LR group and increased in the HBOC-201 group.

Platelet Counts and Other Coagulation Measurements

Platelet counts, fibrinogen, activated partial thromboplastin time, and prothrombin time values were not different in the two groups before and after the treatment.

Clinical Blood Chemistry and Urinalysis

No statistically significant differences between the two groups were reported for alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lipase, bilirubin (total, direct, indirect), or creatinine (Table 4). Blood urea nitrogen concentration was mildly increased in the HBOC-201 group on POD 2 and POD 3 ($P < 0.05$), but because these increases were within normal limits, this finding had no clinical significance. Serum sodium, potassium, chloride, calcium, and glucose values were not different between the two groups at any measured time. There were isolated transient increases in some blood chemistry values, but they were not considered related to exposure to either HBOC-201 or LR. In one patient who received HBOC-201 in a dose of 2 g/kg and underwent reconstruction of an abdominal aortic aneurysm (and superior mesenteric artery), the following enzyme levels were increased: alanine aminotransferase 2382 U/L (POD 2) and 3802 U/L (POD 3); aspartate

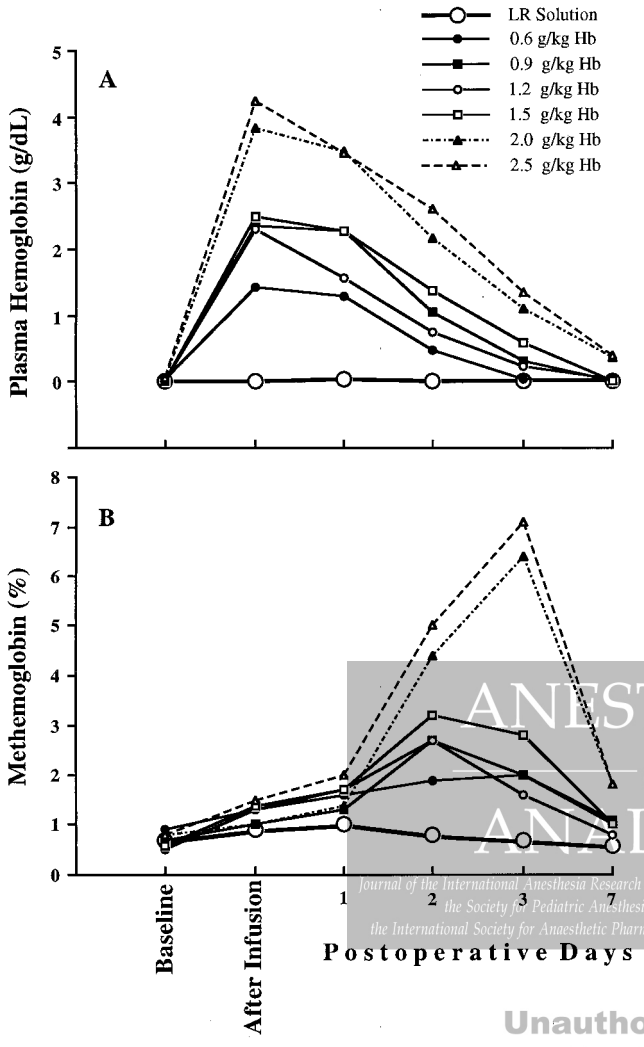

Figure 2. Changes in plasma hemoglobin (Hb) (A) and percent methemoglobin (B) after the infusion of lactated Ringer's (LR) solution or 6 different doses of hemoglobin-based oxygen carrier-201. Data are means for all patients in the respective groups (for number of patients in each group, see Table 1).

aminotransferase 3049 U/L (POD 2) and 6979 U/L (POD 3); lipase 1368 U/L (POD 2) and 576 U/L (POD 3); and lactate dehydrogenase ≥21,500 U/L (both days). All values returned to normal levels before discharge from the hospital, and the patient experienced no other complications. Another patient (who received HBOC-201 in a dose of 0.9 g/kg) had a history of congestive heart failure and underwent hip replacement; his serum lipase concentration increased on POD 2 (406 U/L) and returned to normal on POD 3. Hemoglobin was not detected in the urine of patients who received HBOC-201.

Blood Pressure and Heart Rate

Blood pressure readings (systolic, diastolic, and mean arterial) were slightly but consistently higher in the HBOC-201 group than in the LR group at the end of the surgical procedure and at the time of discharge from the recovery room (Table 5). Average heart rates at the time of transfer from the recovery room were higher in both groups than their respective baseline rates.

Blood Transfusion Requirements

During the entire hospitalization, 21 patients (80.8%) in the LR group received 3.2 ± 1.9 U of blood and 36 patients (85.7%) in the HBOC-201 group received 3.6 ± 3.2 U of blood (includes all units, autologous and allogeneic) ($P = 0.74$). Median blood use in the HBOC-201 and LR groups was 2 (minimum, maximum 1, 20) and 3 (1, 6) units, respectively ($P = 0.82$). However, during surgery only, 17 patients (65.4%) in the LR group received 2.4 ± 1.3 U of blood and 19 patients (45.3%) in the HBOC-201 group received 2.3 ± 1.9 U of blood (includes all units, autologous and allogeneic) ($P = 0.19$).

Discussion

Investigators have long attempted to produce a stroma-free hemoglobin oxygen therapeutic that is nontoxic and has oxygen delivery properties similar to those of the red blood cell. Previous products have produced acute cardiovascular, renal, pancreatic, and coagulation toxicities (15,16). Many of these toxicities were attributed to an inability to purify the hemoglobin and remove contaminants, such as phospholipids and endotoxin (17). HBOC-201 is a highly purified hemoglobin-based oxygen carrier of bovine origin with no detectable endotoxin or phospholipid content. In studies of massive blood loss in dogs and sheep, the administration of large quantities of polymerized bovine hemoglobin resulted in excellent intravascular volume expansion, increased oxygen-carrying capacity, and none of the previously reported toxicities (15–18). In other clinical studies (19–21), the administration of HBOC-201 was not associated with adverse effects on the liver, kidney, pancreas, or coagulation.

Our study, limited to 42 patients, demonstrated that HBOC-201 was well tolerated even when administered in doses up to 245 g. However, it needs to be stressed that only 6 patients received this large (2.5 g/kg) HBOC-201 dose. Preclinical and clinical pharmacologic data indicated that positive effects on oxygen transport physiology could be achieved with HBOC-201 doses that result in plasma hemoglobin concentrations >0.7 g/dL (22,23). The doses selected for this trial were capable of exceeding such levels for approximately 48 to 72 hours (Fig. 2).

No patient deaths occurred during the study. Most of the adverse reactions were not associated with exposure to HBOC-201 or LR but instead represented

Table 4. Serum Enzyme and Other Chemical Variables During the Trial

Variable	LR		HBOC-201		*P* Value (between groups)
	No. of Patients	Mean ± SD	No. of Patients	Mean ± SD	
Alanine aminotransferase (U/L)					
Preoperative baseline	22	32 ± 27	37	27 ± 18	0.38
POD 2	26	31 ± 21	16	186 ± 588	0.18
POD 3	26	28 ± 20	29	168 ± 701	0.31
POD 7 or discharge	24	36 ± 23	35	68 ± 143	0.28
Aspartate aminotransferase (U/L)					
Preoperative baseline	22	27 ± 18	37	24 ± 12	0.49
POD 2	26	47 ± 59	33	176 ± 527	0.22
POD 3	26	44 ± 41	35	255 ± 1171	0.36
POD 7 or discharge	24	31 ± 13	39	45 ± 29*	0.03
Alkaline phosphatase (U/L)					
Preoperative baseline	22	80 ± 24	37	89 ± 51	0.48
POD 2	26	55 ± 18†	4	36 ± 16	0.054
POD 3	26	62 ± 19†	14	56 ± 24*	0.42
POD 7 or discharge	24	87 ± 48	35	91 ± 43	0.70
Lipase (U/L)					
Preoperative baseline	22	100 ± 72	35	86 ± 84	0.52
POD 2	25	113 ± 296*	37	400 ± 764*	0.08
POD 3	25	39 ± 31*	33	116 ± 200	0.06
POD 7 or discharge	24	122 ± 165	37	174 ± 213*	0.32
Bilirubin, total (μmol/L)					
Preoperative baseline	22	13.1 ± 6.7	37	12.6 ± 6.2	0.78
POD 7 or discharge	23	15.8 ± 8.5	34	21.5 ± 14.9†	0.10
Outpatient follow-up	22	11.3 ± 7.4*	36	10.3 ± 4.6†	0.52
Creatinine (mg/dL)					
Preoperative baseline	19	0.94 ± 0.23	36	0.93 ± 0.34	0.92
POD 2	26	1.02 ± 0.25	33	0.81 ± 0.28*	0.003
POD 3	26	0.94 ± 0.25	36	0.89 ± 0.27	0.52
POD 7 or discharge	24	0.93 ± 0.25	38	0.90 ± 0.23	0.61
Blood urea nitrogen (mg/dL)					
Preoperative baseline	19	13.3 ± 4.3	37	13.4 ± 5.3	0.93
POD 2	26	11.2 ± 3.6*	42	15.0 ± 5.6	0.003
POD 3	26	9.2 ± 4.5‡	38	15.2 ± 7.2	0.0004
POD 7 or discharge	24	13.6 ± 7.6	40	13.1 ± 5.5	0.75

All values are averages for all HBOC-201 treatment subgroups.
LR = lactated Ringer's solution, HBOC-201 = hemoglobin-based oxygen carrier-201, POD = postoperative day.
Comparisons within treatments: * $P < 0.05$ versus preoperative baseline; † $P < 0.001$ versus preoperative baseline; ‡ $P < 0.01$ versus preoperative baseline.

possible complications of surgical procedures or underlying disease. Several patients had transient dermatologic changes, and 1 patient with a history of mastocytosis experienced mast cell degranulation after the administration of morphine, a known mast cell degranulator, as well as a test dose of HBOC-201. The complex immune status of mastocytosis patients complicates interpretation of this event. However, we do not know whether IgG-aHBOC-201, which developed in 57.5% of the patients who received HBOC-201, has any potential clinical implications for later repeated use of HBOC-201.

One patient who underwent reconstruction of the aortic and mesenteric arteries and received HBOC-201 (2.0 g/kg) had a large and transient increase in serum transaminases. This clinical picture may represent "acute ischemic hepatitis," which has been described in conjunction with aortic surgery and attributed to splanchnic ischemia (24). It is difficult to associate HBOC-201 administration and this previously described condition. All other blood chemistry values were not statistically significantly different between the two treatment groups. Lee et al. (17) demonstrated that the administration of an early, partially polymerized formulation of bovine hemoglobin (stored frozen) in large doses to rats may induce mild, reversible renal toxicity. The postulated nephrotoxic particles are dimers, products of oxidative inactivation of the chains of unmodified hemoglobin. In our study, creatinine values were not different between the two patient groups; therefore, the previous fear of acute renal failure with acellular hemoglobin solutions may be unfounded for the doses of HBOC-201 used in the present study. In a recent study by LaMuraglia et al. (25), increases in serum creatinine in patients receiving HBOC-201 and LR were similar (average increases, 0.41 and 0.28 in the HBOC-201 and LR groups,

Table 5. Perioperative Hemodynamic Variables

Variable	LR ($n = 26$)	HBOC-201 ($n = 42$)	P Value (between groups)
Blood pressure (mm Hg)			
Systolic			
Before infusion	115 ± 18	125 ± 20	0.36
30 min after infusion	112 ± 19	125 ± 20	0.02
End of operation	123 ± 20*	137 ± 21†	0.01
RR discharge or arrival in ICU	128 ± 18	143 ± 22†	0.01
Diastolic			
Before infusion	67 ± 13	64 ± 14	0.47
30 min after infusion	66 ± 10*	68 ± 14	0.53
End of operation	68 ± 12	75 ± 15†	0.05
RR discharge or arrival in ICU	66 ± 16	77 ± 10†	0.005
Mean arterial			
Before infusion	83 ± 13	83 ± 15	0.98
30 min after infusion	82 ± 13	87 ± 14	0.12
End of operation	86 ± 14	95 ± 15†	0.02
RR discharge or arrival in ICU	87 ± 16*	99 ± 11†	0.002
Heart rate (bpm)			
Before infusion	71 ± 15	69 ± 13	0.43
30 min after infusion	76 ± 18	67 ± 13	0.02
End of operation	77 ± 17	73 ± 16	0.32
RR discharge or arrival in ICU	81 ± 20*	75 ± 15*	0.18

All values are averages for all HBOC-201 treatment subgroups.
LR = lactated Ringer's solution, HBOC-201 = hemoglobin-based oxygen carrier-201, RR = recovery room, ICU = intensive care unit.
Comparisons within treatments: * $P < 0.05$ versus before infusion; † $P < 0.001$ versus before infusion.

respectively; difference not significant). These mild creatinine increases might be attributable to aortic aneurysm reconstruction, which is sometimes associated with alteration of kidney function. However, LaMuraglia et al. (25) found that blood urea nitrogen values significantly increased in patients receiving HBOC-201, presumably because of the HBOC-201-associated high protein load. The absence of hemoglobin in the urine in our study further indicates that HBOC-201 is not eliminated by the kidneys. In fact, primary excretion of HBOC-201 occurs through metabolism in the liver and reticuloendothelial system (21).

Transient skin discoloration occurred rarely and only after the administration of HBOC-201 at larger doses. Possibly, this benign dermatologic effect results from dose-dependent HBOC-201 extravasation into the areas injured by manipulation (shaving, adhesive tapes), because similar occurrences were not reported with exposure to LR solution. Furthermore, neither occasional skin discoloration nor discoloration of serum by HBOC-201 caused problems with pulse oximetry monitoring. The unimpeded ability to monitor pulse oximetry with HBOC-201 in the serum was confirmed previously by Hughes et al. (19).

Circulating methemoglobin in healthy humans is the result of a balance between methemoglobin production (from auto-oxidation and oxidation) and hemoglobin reduction. Methemoglobin, an oxidized form of hemoglobin, is unable to bind oxygen and therefore effectively decreases the oxygen-carrying capacity of hemoglobin. Because enzymes within intact erythrocytes maintain hemoglobin in a reduced state, methemoglobinemia is a concern whenever acellular hemoglobin solutions are given. Mild methemoglobinemia has been described in patients receiving bovine hemoglobin solutions (26). In the present study, the administration of HBOC-201 was followed by an increase in the percent of plasma methemoglobin. Interestingly, the percent of methemoglobin was not high immediately after HBOC-201 infusion but rather had a delayed onset and reached maximal value (mean of all study groups, 3.66%) by POD 3 (Table 3). However, in patients who received the 2.5 g/kg dose of HBOC-201, the mean methemoglobin concentration was 7.1% (Fig. 2). O'Hara et al. (27) demonstrated a somewhat shorter delay, 8 to 12 hours, in maximal methemoglobin concentrations after the infusion of diaspirin cross-linked human hemoglobin. This result indicated that the methemoglobin load was not administered with HBOC-201 but rather was gradually generated by oxidation of plasma hemoglobin. Linberg et al. (28) found that methemoglobin levels below 10% did not significantly alter the delivery of oxygen to organs; consequently, the levels measured in our study probably have little clinical significance. However, the delayed onset of methemoglobinemia coincident with declining concentrations of plasma hemoglobin resulting from the short half-life of HBOC-201 also might decrease the blood oxygen-carrying capacity. The sum of these unwanted effects needs to be closely monitored in patients who are receiving large doses of HBOC-201.

LaMuraglia et al. (25) found small increases in systemic blood pressure in patients who underwent aortic surgery and received HBOC-201. Similarly, in our study, we observed slightly increased blood pressure readings at the end of surgery and the time of discharge from the recovery room in patients who received HBOC-201 (Table 5). This increase was not considered clinically significant, and the mechanism remains obscure. Although nitric oxide binding by the hemoglobin was postulated to be a mechanism of the increase in blood pressure, several recent investigations found that products exhibiting the greatest nitric oxide affinity resulted in the least increase in blood pressure (29). Therefore, another unidentified mechanism may be responsible for the changes in blood pressure occasionally seen after the administration of acellular hemoglobin solutions. It is possible that the improved hemodynamics after HBOC-201 administration are attributable to high intravascular expanding properties of HBOC-201 based on its high colloid-oncotic properties.

There was no difference in estimated blood loss between the two groups, and total hemoglobin concentrations were not different at any measured points between the patients receiving HBOC-201 and those receiving LR. However, the disproportionally large total hemoglobin concentration in relation to the hematocrit on POD 2 in the HBOC-201 group (Fig. 1) may be consistent with one of the following: 1. Patients who received HBOC-201 had more bleeding (lower hematocrit), and the addition of polymerized hemoglobin in the HBOC-201 group may have served to equalize the total hemoglobin levels between the two groups. 2. Patients who received HBOC-201 experienced better intravascular volume expansion than the patients receiving LR. HBOC-201 has high colloid-oncotic pressure (17 mm Hg), which can attract fluid from the interstitial space and lead to a dilutional decrease in hematocrit.

Our study did not show that the use of HBOC-201 reduced the use of blood during the entire hospitalization, primarily, we believe, because of the study design, that is, relatively small single doses in some patients and restriction of the consecutive administration of HBOC-201 when oxygen-carrying capacity was needed postoperatively. The results of another recently published multicenter study demonstrated that HBOC-201 decreased the intraoperative requirements for allogeneic blood transfusion during aortic reconstructive surgery (25). That protocol, the same as ours, did not allow additional HBOC-201 infusion, which resulted in an increased requirement for allogeneic blood transfusion later during the hospitalization. The ultimate result was that the HBOC-201 did not reduce the median allogeneic blood requirements (25).

In conclusion, the intraoperative administration of HBOC-201 to our patients undergoing surgery was generally well tolerated, and even the dose-dependent methemoglobinemia, which reached maximal values by POD 3, did not have a clinically significant impact. The administration of a single dose of HBOC-201 also did not reduce the intraoperative allogeneic blood requirements.

References

1. Dietz NM, Joyner MJ, Warner MA. Blood substitutes: fluids, drugs, or miracle solutions? Anesth Analg 1996;82:390–405.
2. Apstein CS, Dennis RC, Briggs L, et al. Effect of erythrocyte storage and oxyhemoglobin affinity changes on cardiac function. Am J Physiol 1985;248:H508–15.
3. Marik PE, Sibbald WJ. Effect of stored-blood transfusion on oxygen delivery in patients with sepsis. JAMA 1993;269:3024–9.
4. Baele PL, De Bruyere M, Deneys V, et al. Bedside transfusion errors: a prospective survey by the Belgium SAnGUIS Group. Vox Sang 1994;66:117–21.
5. Sloand EM, Pitt E, Klein HG. Safety of the blood supply. JAMA 1995;274:1368–73.
6. Klein HG. Immunomodulatory aspects of transfusion: a once and future risk? Anesthesiology 1999;91:861–5.
7. Nelson CL, Fontenot HJ. Ten strategies to reduce blood loss in orthopedic surgery. Am J Surg 1995;170(6A Suppl):64S–68S.
8. Goodnough LT, Brecher ME, Kanter MH, AuBuchon JP. Transfusion medicine: first of two parts—blood transfusion. N Engl J Med 1999;340:438–47.
9. Lemos MJ, Healy WL. Blood transfusion in orthopaedic operations. J Bone Joint Surg Am 1996;78:1260–70.
10. Spence RK. Surgical red blood cell transfusion practice policies: Blood Management Practice Guidelines Conference. Am J Surg 1995;170(6A Suppl):3S–15S.
11. Breepoel PM, Kreuzer F, Hazevoet M. Interaction of organic phosphates with bovine hemoglobin. I. Oxylabile and phosphate-labile proton binding. Pflugers Arch 1981;389:219–25.
12. Bunn HF. Differences in the interaction of 2,3-diphosphoglycerate with certain mammalian hemoglobins. Science 1971;172:1049–50.
13. Page TC, Light WR, McKay CB, Hellums JD. Oxygen transport by erythrocyte/hemoglobin solution mixtures in an *in vitro* capillary as a model of hemoglobin-based oxygen carrier performance. Microvasc Res 1998;55:54–64.
14. Sprung J, Popp H, O'Hara P, Woletz J. The successful use of hemoglobin-based oxygen carrier as a primary blood substitute during abdominal aneurysm repair with large blood loss. Anesth Analg 2001;92:1413–5.
15. Savitsky JP, Doczi J, Black J, Arnold JD. A clinical safety trial of stroma-free hemoglobin. Clin Pharmacol Ther 1978;23:73–80.
16. Bolin RB, Smith D, Moore G, et al. Hematologic effects of hemoglobin solutions in animals. Prog Clin Biol Res 1983;122:117–26.
17. Lee R, Atsumi N, Jacobs EE, et al. Ultrapure, stroma-free, polymerized bovine hemoglobin solution: evaluation of renal toxicity. J Surg Res 1989;47:407–11.
18. Bosman RJ, Minten J, Lu HR, et al. Free polymerized hemoglobin versus hydroxyethyl starch in resuscitation of hypovolemic dogs. Anesth Analg 1992;75:811–7.
19. Hughes GS Jr, Francom SF, Antal EJ, et al. Hematologic effects of a novel hemoglobin-based oxygen carrier in normal male and female subjects. J Lab Clin Med 1995;126:444–51.
20. Hughes GS, Yancey EP, Albrecht R, et al. Hemoglobin-based oxygen carrier preserves submaximal exercise capacity in humans. Clin Pharmacol Ther 1995;58:434–43.
21. Orringer EP, Gonzalez PM, Hackney AC, et al. A phase I study of polymerized bovine hemoglobin (PBH) in adult patients with sickle cell disease not in crisis at the time of study. In: Beuzard Y, Lubin B, Rosa J, eds. Sickle cell disease and thalassemia: new trends in therapy. Montrogue, France: Colloque INSERM/John Libbey Eurotext Ltd., 1995;234:301–2.

22. Standl T, Horn P, Wilhelm S, et al. Bovine haemoglobin is more potent than autologous red blood cells in restoring muscular tissue oxygenation after profound isovolaemic haemodilution in dogs. Can J Anaesth 1996;43:714–23.
23. Hughes GS, Antal EJ, Locker PK, et al. Physiology and pharmacokinetics of a novel hemoglobin-based oxygen carrier in humans. Crit Care Med 1996;24:756–64.
24. Levy PJ, Tabares AH, Olin JW, et al. Disseminated intravascular coagulation associated with acute ischemic hepatitis after elective aortic aneurysm repair: comparative analysis of 10 cases. J Cardiothorac Vasc Anesth 1997;11:141–8.
25. LaMuraglia GM, O'Hara PJ, Baker WH, et al. The reduction of the allogenic transfusion requirement in aortic surgery with a hemoglobin-based solution. J Vasc Surg 2000;31:299–308.
26. Hughes GS, Francom SF, Antal EJ, et al. Effects of a novel hemoglobin-based oxygen carrier on percent oxygen saturation as determined with arterial blood gas analysis and pulse oximetry. Ann Emerg Med 1996;27:164–9.
27. O'Hara JF, Colburn WA, Tetzlaff JE, et al. Hemoglobin and methemoglobin concentrations after large-dose infusions of diaspirin cross-linked hemoglobin. Anesth Analg 2001;92:44–8.
28. Linberg R, Conover CD, Shum KL, Shorr RG. Hemoglobin based oxygen carriers: how much methemoglobin is too much? Artif Cells Blood Substit Immobil Biotechnol 1998;26:133–48.
29. 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;273:12128–34.