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Does HBOC-201 (Hemopure) Affect Platelet Function in Orthopedic Surgery: A Single-Site Analysis from a Multicenter Study

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HBOC-201, Hemoglobin glutamer-250 (bovine), (Biopure Corp., Cambridge, MA) has been studied in an international, multicenter, pivotal Phase III trial. A subset analysis of use of blood products indicated that the HBOC-201 group required no more than the packed red blood cell (PRBC) group and was limited to less than 6% in both treatment groups. In a subset analysis from one site, platelet function using PFA-100 was assessed before and after transfusion, and compared those receiving HBOC-201 versus PRBC. After initial IRB approval, patient consent for the Phase III trial and blood draws for PFA-100, an additional IRB exemption for retrospective chart review was obtained. cEPI and cADP means were compared at seven time periods: true baseline(before starting surgery and anesthesia), before transfusion, after transfusion, 1 day, 2 days , 3 to 9 days and 21 or more days after transfusion. Twenty-seven (HBOC: n = 12, PRBC: n = 15) subjects were studied. Comparing data from before transfusion and baseline did not show statistically significant differences in any of cEPI or cADP measurements. cEPI means for the HBOC-201 group increased after transfusion compared to the true baseline ($P = 0.01$), before transfusion ($P = 0.0004$) and day 1 after transfusion ($P = 0.002$). cADP means for the HBOC-201 group were greater after transfusion compared to the true baseline ($P = 0.05$) and before transfusion ($P = 0.005$). In the PRBC group there were no significant difference in cEPI and cADP means between all of the time periods. Our study shows that HBOC-201 causes mild platelet dysfunction. Although there were significant changes after HBOC infusion and cEPI and cADP mean values were above the upper normal limit, they did not reach the non-closure time. Further controlled studies are needed to establish definitively the effects that HBOC-201 has on platelet function in patients.

Keywords: hemoglobin-based oxygen carrier, HBOC-201, Hemopure, hemoglobin–glutamer 250 (bovine), platelet dysfunction, PFA-100

INTRODUCTION

HBOC-201 (Hemopure; Hemoglobin–glutamer 250 [bovine]; Biopure Corp., Cambridge, MA) is a purified cell-free gluteraldehyde crosslinked and polymerized bovine hemoglobin (Hb) in a modified lactated Ringer's solution with: 13 ± 1g/dL Hb (30–35 g Hb/250 mL unit), pH 7.6 to 7.9, P_{50} = 40 mmHg. HBOC-201 can be stored at room temperature (range, 2–30°C) for up to 3 years, does not require crossmatching, has an oxygen release that is independent of 2, 3-diphosphoglycerate,

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and has a circulatory half-life of 19 hours.[1] Study of HBOC-201 was completed in an international, multicenter, pivotal Phase III trial and has been shown to prevent packed red blood cell (PRBC) transfusion in 59.1% of patients undergoing orthopedic surgery by the sixth week.[1] The use of other allogenic blood components such as platelets, cryoprecipitate, and albumin was limited to less than 6% of patients in both treatment groups.[2] HBOC-201 was approved by the South Africa Medicines Control Council for acutely anemic adult surgical patients in 2001, and a veterinary version (Hemoglobin glutamer-200 [bovine]; (Oxyglobin) solution was approved by the US Food and Drug Administration and the European Union for canine anemia in 1998 and 1999, respectively.[3]

In an animal study on the effects of HBOC-201 on platelet function, it was demonstrated that animals that were resuscitated with HBOC-201 or hetastarch 6% both had significant increases in the platelet function analyzer closure time (PFA-CT) initially. This change returned to normal in the hetastarch group by 24 hours, but reached a maximum in the HBOC-201 group at 24 hours and normalized in 72 hours after transfusion.[4]

We assessed platelet function before and after HBOC-201 transfusion using PFA-100 in a subset analysis from one site of a large multicenter study and compared it with those receiving PRBC in the same site.[5]

METHODS

After UC Davis Institutional Review Board approval, patients were asked to participate in the HEM 0115 HBOC-201 trial. If they agreed to participate, after signing consent, they were also asked if they would be interested in participating in a single-site companion study involving additional blood draws to evaluate platelet function before, during, and after treatment periods. The subject then signed a second UC Davis Institutional Review Board-approved additional consent for platelet function blood draws. Because this study was completed in 2000, an additional UC Davis Institutional Review Board exemption was obtained in 2006 to review subject charts of those participating. Then, it was determined to which group the subject was randomized because the platelet function laboratory was blinded to the groups during the course of the study. The whole trial study population[1] included male and female (nonpregnant/lactating women) patients, 18 years or older, undergoing non-emergency orthopedic surgery. Patients who did not receive recombinant erythropoietin, predonated blood, or had been scheduled for normovolemic hemodilution were included. Patients were expected to require at least two units of PRBC transfusion before midnight of postoperative day 3. Patients were randomized to HBOC-201 or PRBC at the first transfusion decision based on the investigator's assessment of the transfusion need and a total blood Hb of less than 10.5 g/dL. Subsequent transfusion decisions required a total Hb of less than 10.5 g/dL and the patient having at least one of the following clinical signs: heart rate 100 beats per minute or greater; systolic blood pressure less than 90 mmHg or less than 70% of preoperative screening value; electrocardiogram evidence of myocardial ischemia; metabolic acidosis; acute blood loss (more than 7 mL/kg within 2 hours or less); oliguria with urine output less than 0.5 mL/kg/h for at least 2 hours; and restricted patient activity as a result of weakness or dizziness. Once treatment started, additional treatment was permitted for up to 6 days using the same criteria. In addition, there could be crossover to treatment with PRBC based on the investigator's assessment of clinical need. Only those patients who received either HBOC-201 or PRBC during or after orthopedic surgery were included in this subset analysis; those who crossed over to PRBC, thus receiving both PRBC and HBOC-201, and those who did not require transfusion were excluded from the subset analysis. The crossover group (HBOC + PRBC) was heterogeneous and thus was not included in our study. This group consisted of those patients who received PRBC after reaching the maximum allowed units of HBOC, the maximum 6-day treatment time period, or after receiving the physician or subject's request to switch.[1]

Platelet function was measured using the PFA-100 system (Dade Behring, Marburg, Germany). It is a rapid screening tool that has been proven to be more sensitive in detecting major platelet function defects than the standard bleeding time measurement method.[5] The PFA-100 simulates high shear stress conditions in vivo using a capillary apparatus to measure platelet function. The PFA-100 measures the closure time (CT), which is the time when blood ceases to flow through the apparatus as a result of the formation of a platelet thrombus. Platelet aggregation and adhesion may be the result of the presence of platelet agonists and interactions with the collagen-coated membrane. Two types of agonists are used to coat the collagen cartridges, epinephrine (cEPI) or ADP (cADP).[5,6] The maximum allowable CT was 300 seconds, and any time longer than this was reported as nonclosure (NC). The normal reference ranges were 71 to 118 seconds for cADP and 94 to 193 seconds for cEPI.[7] Blood samples were obtained from all subjects by venipuncture and were collected in 3-mL tubes containing 3.2% sodium

citrate and assay was performed in duplicate according to the manufacturer's instructions.

We assessed platelet function at seven time periods: true baseline, before transfusion, after transfusion, 1 day after transfusion (Day 1 A/T), 2 days after transfusion (Day 2 A/T), 3 to 9 days after transfusion (Day 3–9 A/T), and 21 or more days after transfusion (Day 21 and after, and compared the two treatment groups, HBOC and PRBC. True baseline values were measured before any transfusion or surgery. Before transfusion values were measured before transfusion and after the start of surgery. Posttransfusion values were measured after transfusion and after the start of surgery. We also had additional short-term "Day 3–9 A/T" and long-term "Day 21 and after" follow-up data.

Statistical analysis

To compare mean cEPI and cADP values, we used an unbalanced repeated measures analysis of variance (ANOVA) model with time period and treatment group and their possible interaction as fixed effects and patient as a random effect. The adjusted means were estimated from the repeated measures ANOVA. The model-adjusted means are less biased because the repeated-measures ANOVA model takes into consideration the correlation of observations across time periods to impute missing values. They are completely unbiased when missing data are missing at random.

The analysis was performed using SAS Procedure MIXED, Version 9.1 (SAS Inc, Cary, NC).

RESULTS

Forty-one subjects enrolled in the clinical trial study at the UC Davis site (HBOC n = 12, PRBC n = 15, both HBOC + PRBC n = 6, and no transfusion n = 8), but only those who received either HBOC or PRBC were included in our subset analysis (n = 27). There were no significant differences in the baseline mean values between HBOC and PRBC groups for both cartridges (cEPI: $P = 0.2$, cADP: $P = 0.5$) (Tables 1 and 2).

cEPI means for the HBOC group were significantly greater in the "after transfusion" time period compared with "true baseline" ($P = 0.01$), "before transfusion" ($P = 0.0004$), and "Day 1 A/T" ($P = 0.002$) (Tables 1 and 3). There were also data for "Day 3–9 A/T" and "Day 21 and after" follow ups; however, because the patients had already been discharged by those times, there were fewer observations for those time periods. cEPI values were measured on "Day 3–9 A/T" for no subjects in the HBOC group and three subjects in the PRBC group, and values were measured on "Day 21 and after" for four subjects in HBOC and five subjects in PRBC. Data on "Day 21 and after" showed significantly greater cEPI means compared with "true baseline" ($P = 0.02$), "before transfusion" ($P = 0.002$), and "Day 1 A/T"

Table 1. cEPI and cADP adjusted mean and median by treatment group and time period.

		cEPI					cADP				
Group	Period	n	Adjusted mean	Median	Standard error	Standard deviation	n	Adjusted mean	Median	Standard error	Standard deviation
HBOC	True BL	3	127.5	146.0	38.0	32.3	3	102.8	98.0	17.0	21.7
HBOC	Before transfusion	10	119.8	116.0	21.4	44.0	7	98.8	83.0	11.6	36.9
HBOC	After transfusion	4	259.4	260.5	33.1	45.4	3	150.8	110.0	16.7	62.9
HBOC	Day 1 A/T	3	101.7	93.0	34.8	21.4	2	112.6	95.0	20.2	17.0
HBOC	Day 2 A/T	2	188.5	202.0	45.6	138.6	2	101	101.0	20.4	28.3
HBOC	Day 21 and after	4	237.9	250.0	32.9	72.7	4	64.7	73.5	15.1	9.3
PRBC	True BL	5	195.1	167.0	29.5	72.5	5	87.1	75.0	13	39.5
PRBC	Before transfusion	11	187.5	178.0	20.6	66.3	7	97.9	96.0	11.4	35.3
PRBC	After transfusion	3	178.5	101.0	38.1	122.4	3	75.5	66.0	16.6	25.92
PRBC	Day 1 A/T	3	211.7	274.0	34.2	106.6	4	109.2	110.0	13.6	15.26
PRBC	Day 2 A/T	4	188.3	217.5	32.8	119.0	3	79.5	74.0	15.9	27.0
PRBC	Day 3–9 A/T	3	182.5	119.0	30.1	100.4	2	78	69.5	16.0	10.4
PRBC	Day 21 and after	5	142.6	118.0	29.6	52.7	5	96.4	78.0	13.1	28.9

HBOC, hemoglobin-based oxygen carrier-201; PRBC, packed red blood cell; Adjusted Mean, adjusted means under the repeated-measure analysis of variance model (see "Methods"); True BL, true baseline (before start of surgery or anesthesia); Before transfusion, before transfusion (after start of surgery or anesthesia); After transfusion, after transfusion on the day of surgery; Day 1 A/T, 1 day after last transfusion; Day 2 A/T, 2 days after last transfusion; Day 3–9 A/T, 3 to 9 days after last transfusion; Day 21 and after, 21 or more days after last transfusion.

Table 2. cEPI and cADP between-group mean differences (HBOC-PRBC) at the same time period.

	cEPI			cADP		
Period	Mean difference	Standard error	*P* value	Mean difference	Standard error	*P* value
True BL	–67.6	48.0	0.170	15.7	21.4	0.473
Before transfusion	–67.7	29.7	0.030	0.9	16.3	0.957
After transfusion	80.9	50.4	0.120	75.4	23.5	0.004
Day 1 A/T	–109.9	48.8	0.030	3.4	24.3	0.889
Day 2 A/T	0.2	56.1	0.997	21.4	25.8	0.417
Day 21 and after	95.3	44.3	0.040	–31.7	19.9	0.128

HBOC, hemoglobin-based oxygen carrier-201; PRBC, packed red blood cell; True BL, true baseline (before the start of surgery or anesthesia); Before transfusion, before transfusion (after the start of surgery or anesthesia); After transfusion, after transfusion on day of surgery; Day 1 A/T, 1 day after last transfusion; Day 2 A/T, 2 days after last transfusion; Day 21 and after, 21 or more days after last transfusion.

($P = 0.005$). In the PRBC group, there were no significant differences between all of the time periods (Tables 1 and 3). Comparing cEPI mean values between HBOC and PRBC groups showed a significant difference in the "before transfusion" time period ($P = 0.003$; HBOC = 119 seconds, PRBC = 187 seconds) and "Day 1 A/T" ($P = 0.03$; HBOC = 101 seconds, PRBC = 211 seconds) and "Day 21 and after" ($P = 0.04$; HBOC = 237 seconds, PRBC = 142 seconds) (Table 1 and Figure 1).

cADP mean values for the HBOC-201 group were significantly greater "after transfusion" compared with true baseline ($P = 0.05$) and "before transfusion" ($P = 0.005$) (Tables 1 and 3). cADP values were measured on "Day 3–9 A/T" for no subjects in the HBOC group and two subjects in the PRBC group, and values were measured on "Day 21 and after" for four subjects in the HBOC group and five subjects in the PRBC group. cADP means on "Day 21 and after" were lower

Table 3. cEPI and cADP *P* values for mean comparisons within groups among time periods.

Period	Versus period	HBOC-cEPI *P* value	HBOC-cADP *P* value	PRBC-cEPI *P* value	PRBC-cADP *P* value
True BL	Before transfusion	0.85	0.84	0.82	0.47
True BL	After transfusion	0.01	0.05	0.72	0.55
True BL	Day 1 A/T	0.61	0.70	0.68	0.16
True BL	Day 2 A/T	0.28	0.94	0.87	0.68
True BL	Day 3–9 A/T	—	—	0.74	0.62
True BL	Day 21 and after	0.03	0.08	0.18	0.56
Before transfusion	After transfusion	0.0004	0.006	0.83	0.23
Before transfusion	Day 1 A/T	0.63	0.48	0.53	0.47
Before transfusion	Day 2 A/T	0.16	0.92	0.98	0.29
Before transfusion	Day 3–9 A/T	—	—	0.89	0.29
Before transfusion	Day 21 and after	0.002	0.07	0.18	0.92
After transfusion	Day 1 A/T	0.002	0.13	0.49	0.09
After transfusion	Day 2 A/T	0.21	0.07	0.83	0.84
After transfusion	Day 3–9 A/T	—	—	0.92	0.89
After transfusion	Day 21 and after	0.63	0.0004	0.45	0.31
Day 1 A/T	Day 2 A/T	0.12	0.68	0.57	0.11
Day 1 A/T	Day 3–9 A/T	—	—	0.47	0.10
Day 1 A/T	Day 21 and after	0.005	0.06	0.12	0.44
Day 2 A/T	Day 3–9 A/T	—	—	0.88	0.94
Day 2 A/T	Day 21 and after	0.34	0.09	0.28	0.37
Day 3–9 A/T	Day 21 and after	—	—	0.34	0.35

HBOC, hemoglobin-based oxygen carrier-201; PRBC, packed red blood cell; True BL, true baseline (before the start of surgery or anesthesia); Before transfusion, before transfusion (after the start of surgery or anesthesia); After transfusion, after transfusion on the day of surgery; Day 1 A/T, 1 day after last transfusion; Day 2 A/T, 2 days after last transfusion; Day 3–9 A/T, 3 to 9 days after last transfusion; Day 21 and after, 21 or more days after last transfusion.

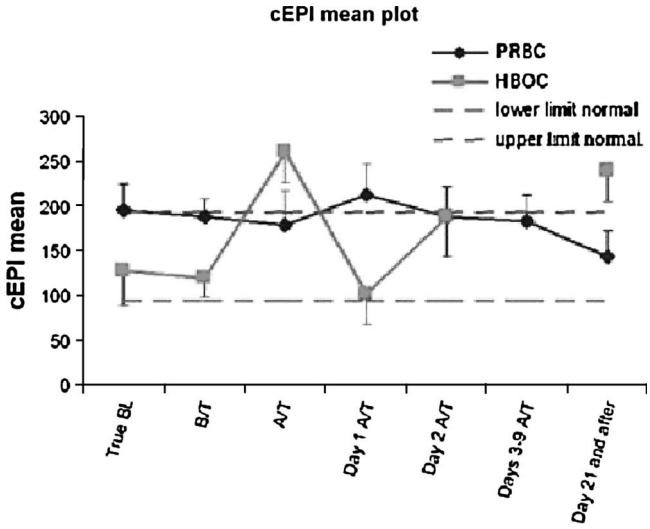

FIGURE 1. Adjusted cEPI means were compared in two treatment groups, HBOC-201 and PRBC. In the HBOC-201 treatment group, there was a significant increase in cEPI after transfusion (true BL versus A/T, $P = 0.01$; B/T versus A/T, $P = 0.0004$; A/T versus Day 1 A/T, $P = 0.001$). There were no data on days 3 to 9 after transfusion in the HBOC group and there were also limited data present for Days 21 and after (HBOC n = 4 and PRBC n = 5). Mean cEPI values significantly increased on Day 21 compared with true baseline ($P = 0.02$), before transfusion ($P = 0.002$), and Day 1 after transfusion ($P = 0.004$). For the PRBC group, there was no significant difference among all of the time periods. True BL, true baseline (before start of surgery or anesthesia); B/T, before transfusion (after start of surgery or anesthesia); A/T, after transfusion on day of surgery; Day 1 A/T, 1 day after last transfusion; 2 days A/T, 2 days after last transfusion; 3–9 days A/T, 3 to 9 days after last transfusion; Day 21 and after, 21 or more days after last transfusion.

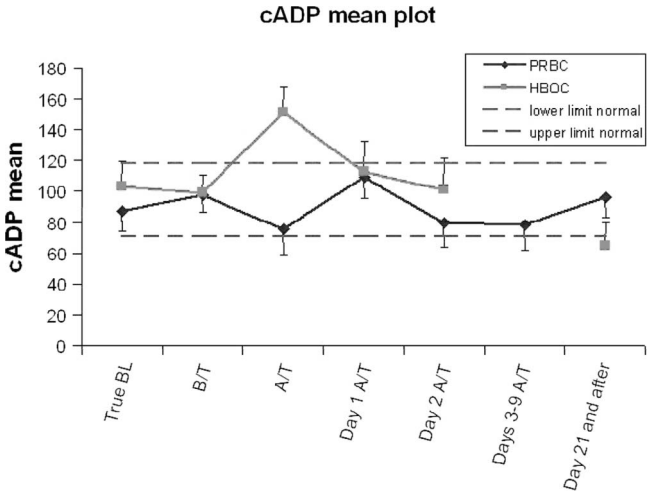

FIGURE 2. Adjusted cADP means were compared in two treatment groups, HBOC-201 and PRBC. In the HBOC-201 treatment group, there was a significant increase in cADP after transfusion (true BL versus A/T, $P = 0.05$; B/T versus A/T, $P = 0.005$). There were no data on Days 3–9 after transfusion for the HBOC group and there was also limited data present for Day 21 and after (HBOC n = 4 and PRBC n = 5). Mean cADP values decreased on Day 21 compared with true baseline ($P = 0.07$), before transfusion ($P = 0.06$), and Day 1 after transfusion ($P = 0.06$). For the PRBC group, there were no significant differences among all of the time periods. A significant difference between HBOC and PRBC groups in A/T existed ($P = 0.004$). True BL, true baseline (before start of surgery or anesthesia); B/T, before transfusion (after the start of surgery or anesthesia); A/T, after transfusion on day of surgery; Day 1 A/T, 1 day after last transfusion; 2 days A/T, 2 days after last transfusion; 3–9 days A/T, 3 to 9 days after last transfusion; Day 21 and after, 21 or more days after last transfusion.

compared with "true baseline" ($P = 0.08$), "before transfusion" ($P = 0.07$), and "Day 1 A/T" ($P = 0.06$). In the PRBC group, there were no significant changes in cADP means among all time periods (Table 3). When comparing cADP mean values between HBOC and PRBC groups, there is a significant difference in the "after transfusion" time period ($P = 0.004$) (Table 2 and Figure 2).

DISCUSSION

With the approval of HBOC-201 for human use in South Africa and the completion of its multinational Phase III trial, studying the possible adverse coagulation effects of HBOC-201 is necessary. There have been numerous studies involving HBOC-201, but none analyzing its effects on human platelet function. Because most patients in need of HBOCs experience hemorrhage resulting from trauma or major surgery, platelet function is important in preventing further blood loss.

In an animal study, authors demonstrated that HBOC-201 and hetastarch 6% interfere with platelet function in swine by increasing PFA-CT compared with animals that did not receive transfusion. These effects resolved at 24 hours in the hetastarch group, but the HBOC group remained hypocoagulopathic at 24 hours and did not normalize until 48 hours after blood transfusion.[4] Gelatin and HBOC-201 similarly induced a significant reduction in arterial thrombosis formation manifested by significantly decreased cyclic flow reductions (from median of 7 to 1 and 6 to 1, respectively) and increased bleeding time (from 88–98 seconds and 81–102 seconds, respectively; $P < 0.05$) in another animal study.[8] Ex vivo comparisons of coagulation interference of two different molecular weight HBOCs (OxyVita, a new-generation zero-linked polymerized

bovine hemoglobin-based oxygen carrier, 33 megadalton and HBOC-200, 200 kilodalton) and hetastarch 6% (670 kilodalton) with normal saline, using thrombelastography, showed that the two HBOCs decreased clot strength (MA and G) at low and medium dilutions. The magnitude of this effect was equivalent to that of 6% hetastarch but was significantly greater than that of normal saline solution.[9] In contrast, a study of another hemoglobin-based oxygen carrier on rabbits showed that o-Raffinose polymerized hemoglobin (Hemolink; Hemosol Inc., Toronto, Ontario, Canada) ameliorates the hemostatic defect associated with anemia and thrombocytopenia. This effect persisted at least 24 hours after a single bolus infusion.[10]

In PFA-100 measurements, the cEPI cartridge is more sensitive overall (86%) than the cADP cartridge (81%) and is especially sensitive to drug-related platelet dysfunction.[11] The clinical predictive value of PFA-100 is debatable, because severe bleeding caused by thrombocytopenia is rare and occurs only in patients with a concomitant coagulopathy or anatomic defects in the vascular system.[12] In terms of inherited platelet defects or von Willebrand Disease, it appears evident that a simultaneous increase in CEPI-CT and CADP-CT carries a risk for clinical bleeding. Similarly, bleeding is a relevant adverse effect of GPIIb/IIIa inhibitors, which prolong both CEPI-CT and CADP-CT mostly greater than 300 seconds (NC). Hence, NC, particularly when it occurs with both cartridges, can be considered a relevant risk factor for bleeding.[12] Platelet counts and hematocrits below 8×10^9/L and 30%, respectively, cause significant prolongation of the closure time.[13] One study showed that cEPI-CT increased more (60%) than cADP-CT (25%) when hematocrit levels decreased from 40% to 20% in vitro.[13]

Anesthetic drugs have some effects on platelet function, because multiple in vitro and in vivo studies demonstrate that halothane, sevoflurane, and propofol inhibit platelet function in a reversible and dose-related manner at concentrations used clinically.[14] There was a residual suppressive effect 1 hour postoperatively with sevoflurane and propofol.[15] Certain foods are known to inhibit platelet function as well such as fatty acids and cocoa.[12] Although a true control group was lacking in our study, by comparing the baseline values with the ''before transfusion'' time period, it was demonstrated that anesthetic drugs or surgery had no significant effect on PFA-CT. Perhaps the reason for this is because the delayed effect of the anesthetic drugs or the drugs used did not have any platelet function effects. The anesthetic in our study was left up to investigators' preference; however, because this was a single-site study with all anesthetics supervised by one anesthesiologist (J.S.J.), it is unlikely that there were major differences in anesthetic drugs or techniques, hence unlikely interference with our results. Also, in the multicenter trial results,[1] the average total Hb and hematocrits were lower in the HBOC treatment group compared with the PRBC group through 5 days after transfusion. The mean plasma Hb concentration was 1.80 ± 0.05 g/dL after the final HBOC-201 infusion and decreased consistently with a half-life of 19 hours; at 6 weeks follow up, plasma Hb values were negligible.[1] Analysis of total Hb concentrations as a function of the number of infusions indicates that patients requiring ongoing HBOC-201 treatment maintained a lower total Hb concentration than those given PRBCs. The difference was statistically significant at any pre- and post-infusion point.[1] This may explain the significant results in cEPI we obtained for our HBOC group.

Cell-free Hb is prone to auto-oxidation to methemoglobin; HBOC-201 infusion in surgical patients demonstrated that the percent of plasma methemoglobin increased in a dose-dependent manner with a delayed onset and reached maximal value 3 days after transfusion. The mean value of methemoglobin concentration was 3.66% and in patients who received high doses of HBOC-201 (2.5 g/kg), the mean was 7.1%.[16] In the process of oxidation, reactive oxygen species are generated. Platelets contain several glycoprotein receptors with thiol groups and vicinal thiols, making them redox-sensitive structures. These glycoprotein receptors include adhesion receptor glycoprotein IIb/IIIa and the P2Y12 ADP receptor, which are involved in platelet aggregation and activation. Disulfide isomerase such as protein disulfide isomerase, which has a role in platelet aggregation, is another redox-sensitive structure. A redox homeostasis exists in blood as a result of a transplasma membrane redox system of platelets, which can be impaired by free radicals.[17]

The ANOVA parametric assumptions were confirmed using diagnostic plots. To evaluate constant variance, we constructed plots of residuals on the Y axis versus predicted values on the X axis. The random pattern of data points for each range of predicted value indicated that there were no major violations of the constant variance assumption. To confirm normality, we used residual quantile–quantile plots. The data points did not significantly deviate from the reference line indicating that the parametric normality assumption was met.

The authors acknowledge that this study has a number of limitations. These include, but are not limited to, first, a number of subjects' data could not be evaluated, because the data set was incomplete, especially in short-term and long-term follow up (not all time periods had PFAs drawn in each subject). Collecting and sending the samples to a specialized

laboratory is challenging, especially given that many of the subjects' follow-up visits occurred off-site and sometimes in subjects' homes or local doctors' offices. The crossover group, in which subjects received both treatments (HBOC + PRBC), and the group in which patients who did not receive any transfusion, were all excluded from the analysis. This first subset was complicated by the fact that there were multiple reasons for entering this group such as exceeding the time limit on HBOC infusions and/or reaching the maximum units of HBOC infused. However, subjects had baseline PFA samples drawn before either anesthesia or any HBOC or PRBC infused/transfused, so there should not be any bias here, and because samples were compared with baseline, unlikely interference from number of units of HBOC infused. Additionally, as a result of the nature of the Institutional Review Board approval for this study, it was not possible to link amounts of HBOC or PRBC that the subjects received and correlate with the PFA data as well as not being able to correlate other laboratory results. The subjects signed consents to have extra blood drawn for the purpose of PFA analysis. This important limitation may have an influence on the results; however, because there were 27 subjects whose data was tabulated for this study, it is reasonable to assume that the two groups were relatively equivalent in terms of PRBC or HBOC used. The hematocrits are likely lower in the HBOC group early, but equalized by 6 weeks.[1] It may be possible that the effect on platelet function seen in this study was the result of hemodilution rather than the HBOC itself. The hemodilution, although present early on in the course of the study was resolved, usually by day 5 to 6, so changes resulting from this would likely be eliminated longer term.[1] The laboratory measuring the PFA-CT was blinded; however, retrospective chart review of the patients' data was not blinded

We conclude that PRBC has no significant effect on platelet function. On the other hand, HBOC-201 causes platelet dysfunction, which reversed after approximately one HBOC-201 half-life on "Day 1 A/T." Because there were insufficient data for the follow-up time periods, we were not able to come to a conclusion for these time periods. However, it can be noted that there is a trend in HBOC having short-term and long-term effects on platelet function. Because the cEPI cartridge is more sensitive to drug-induced platelet dysfunction, there were more significant deviations from the normal values with the cEPI measurements.

In summary, our study shows that HBOC has mild platelet dysfunction effects. Although there were significant changes after HBOC-201 infusion and cEPI and cADP mean values were above the upper normal limit, they did not reach the NC time. Further controlled studies are needed to clearly establish the effects that HBOC has on platelets in patients.

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