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Safety of Bioplasma FDP and Hemopure in rhesus macaques after 30% hemorrhage

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ABSTRACT

Objectives Prehospital transfusion can be life-saving when transport is delayed but conventional plasma, red cells, and whole blood are often unavailable out of hospital. Shelf-stable products are needed as a temporary bridge to in-hospital transfusion. Bioplasma FDP (freeze-dried plasma) and Hemopure (hemoglobin-based oxygen carrier; HBOC) are products with potential for prehospital use. In vivo use of these products together has not been reported. This study assessed the safety of intravenous administration of HBOC+FDP, relative to normal saline (NS), in rhesus macaques (RM).

Methods After 30% blood volume removal and 30 minutes in shock, animals were resuscitated with either NS or two units (RM size adjusted) each of HBOC+FDP during 60 minutes. Sequential blood samples were collected. After neurological assessment, animals were killed at 24 hours and tissues collected for histopathology.

Results Due to a shortage of RM during the COVID-19 pandemic, the study was stopped after nine animals (HBOC+FDP, seven; NS, two). All animals displayed physiologic and tissue changes consistent with hemorrhagic shock and recovered normally. There was no pattern of cardiovascular, blood gas, metabolic, coagulation, histologic, or neurological changes suggestive of risk associated with HBOC+FDP.

Conclusion There was no evidence of harm associated with the combined use of Hemopure and Bioplasma FDP. No differences were noted between groups in safety-related cardiovascular, pulmonary, renal or other organ or metabolic parameters. Hemostasis and thrombosis-related parameters were consistent with expected responses to hemorrhagic shock and did not differ between groups. All animals survived normally with intact neurological function.

Level of evidence Not applicable.

WHAT IS ALREADY KNOWON THIS TOPIC

⇒ Hemopure is a hemoglobin-based oxygen carrier that is used to treat patients with severe, life-threatening anemia for whom blood transfusion is indicated, but not an option.
⇒ Hemopure is approved for medical use in South Africa and is used under an expanded access (compassionate use) IND in the United States.
⇒ Bioplasma FDP is a freeze-dried plasma used as an alternative to fresh frozen plasma in South Africa.

WHAT THIS STUDY ADDS

⇒ This is the first in vivo study using Hemopure and Bioplasma FDP together. It shows that the products can be used safely together in nonhuman primates.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Findings of this study are supportive of a potential clinical study using Hemopure and Bioplasma FDP to treat patients with hemorrhagic shock in the out-of-hospital setting.

BACKGROUND-

Recent studies have demonstrated that prehospital administration of blood products (plasma, red cells, whole blood) is associated with improved survival in patients with traumatic hemorrhage.[1–3] The benefit appears greatest when patients cannot reach a center for in-hospital transfusion within approximately 30–40 minutes of injury.[2 4] Owing to storage and laboratory constraints, it is expected that many patients in remote, disaster, or military settings would not have timely access to conventional blood products. There remains a significant unmet medical need for shelf-stable alternatives to standard blood products, such as dried plasma and a universal oxygen carrier, for use in prehospital and austere environments when access to in-hospital transfusion may be delayed.[5 6]

Freeze-dried plasmas (FDPs) have been available in South Africa, France, Germany, and a number of other countries since the 1990s.[7] In recent years, many countries have reported successful prehospital use of dried plasma for patients with severe bleeding.[7] Bioplasma FDP is a pooled, solvent/detergent pathogen reduced, freeze-dried plasma that has been in clinical use in South Africa since 1996, with a strong safety record.[8] Consensus guidelines for blood management in South Africa state that the product is used interchangeably with fresh frozen plasma in all types of patients.[9 10]

Hemoglobin-based oxygen carriers (HBOCs) have been used clinically to provide oxygen-carrying capacity when red cells are not available, as a bridge to transfusion, or when red cells are not acceptable (eg, Jehovah's Witness patients).[11 12] One HBOC, Hemopure, was approved for use in

South Africa in the early 2000s and has also been available under expanded access (compassionate use) in the USA and other countries. Levien reviewed[13] a hemovigilance program of 336 patients in South Africa who received Hemopure, and found that patients responded well clinically with no pattern of Hemopure-attributable significant adverse events. No deaths were either probably or definitely attributable to Hemopure.[13] A review of use of Hemopure in 1701 patients (including clinical trials, compassionate use, and clinical use) showed no increase in adverse events related to the product.[14] South African clinical experts have published usage guidelines.[15]

South Africa is the only country in the world that has both an FDP and an oxygen carrier approved for medical use. Considering the burden of trauma in South Africa, there appears to be great potential for use of these products together in situations where transport of patients with severe hemorrhage for in-hospital transfusion may be delayed.[16 17] To date, there have been no reports in the literature on the safety of using both products together, either in animal or human studies. There are no known mechanisms of potential drug interaction between these two products. Nonetheless, as unforeseen interactions cannot be ruled out, an animal study was thought to be useful to confirm in vivo compatibility of these products. This could facilitate a future clinical trial that includes both products.

Old World monkeys and man have cell surface glycoproteins that differ from most other animals, making it impossible to find any other experimental species in which the animal's native red cells would be compatible with infused human plasma.[18] Xeno incompatibility is a barrier to translation of human blood products for hemorrhage in large animal models, with the exception of non-human primates (NHPs).[19] Although Hemopure is compatible across many species, Bioplasma FDP (human plasma) is not. Therefore, to be able to test the actual 'off the shelf' products in question, an NHP model is necessary. We used a model of severe hemorrhagic shock in rhesus macaques to evaluate the safety of resuscitation using Bioplasma FDP and Hemopure. This study was conducted to determine the safety of intravenous administration of Hemopure and Bioplasma FDP simultaneously or sequentially, relative to normal saline (NS), in rhesus macaques after significant hemorrhage.

MATERIALS AND METHODS

All procedures were performed in an Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited facility. Animals were assigned randomly to treatment group. Investigative teams were blinded to treatment. Veterinary staff performing the necropsy, histological assessments, ECG interpretation, and final neurological assessments were blinded to treatment.

Animal preparation: male rhesus macaques (*Macaca mulatta*) weighing 8–14 kg (age approximately 7–10 years) were single-housed, acclimated for 35 days prior to study, and underwent a complete veterinary health assessment. All animals were confirmed negative for herpes B, simian T-lymphothrophic virus (STLV), simian immunodeficiency virus (SIV), simian retrovirus (SRV), and tuberculosis. Animals were anesthetized and instrumented as previously described.[19] Feed was withheld 12 hours before surgery. Animals were premedicated with Buprenex (0.03 mg/kg; Reckitt & Colman Pharmaceuticals Inc., Richmond, VA) and Telazol (3.0 mg/kg; Zoetis Inc., Kalamazoo, MI), and weighed (Detecto Scale, Webb City, MO). Airway intubation and control was achieved with 4–5.5 mm endotracheal tubes (Rusch–Teleflex, Research Triangle Park, NC) and mechanical ventilation instituted at 12–15 breaths/min, with a fractional inspired oxygen of 21% to 25% and isoflurane (0.8–2.0%) inhalational anesthesia. The femoral arteries were cannulated with 5 Fr catheters (Teleflex, Research Triangle Park, NC) for controlled hemorrhage (left) and continuous blood pressure monitoring (right). The right femoral vein was cannulated with a 7 Fr triple-lumen catheter (Cook Medical, Bloomington, IN) for intravenous infusion. Arterial blood pressure was monitored using a Drager Apollo Anesthesia Workstation. A 12-lead ECG was used to monitor heart rate (HR) and arrhythmia development continuously through 240 minutes and interpreted by a board-certified veterinarian. Core body temperature was monitored continuously via a 10 Fr rectal temperature probe and maintained between 36.0°C and 38.0°C throughout the period of anesthesia with blankets, heat packs, and Bair Hugger Model 750 (Arizant Inc., Eden Prairie, MN) as required.

The experimental timeline is shown in figure 1. After a 30-minute stabilization period, animals underwent a controlled arterial hemorrhage to remove 30% of estimated blood volume (EBV) during a 5-minute period. A blood collection bag containing anticoagulant citrate phosphate dextrose adenine solution at a 1:10 ratio was attached to the femoral arterial catheter and placed on a DCM3000 Data Collection Mixer (Genesis BPS, Ramsey, NJ). The stopcock on the catheter was opened allowing free bleeding into the bag. Rhesus macaque EBV was estimated at 55 mL/kg.[20] When a blood loss of 30% EBV was achieved, the stopcock was closed. This produced a class III hemorrhage.[21] After blood withdrawal, animals underwent a 30-minute period of hemorrhagic shock prior to the resuscitation phase.

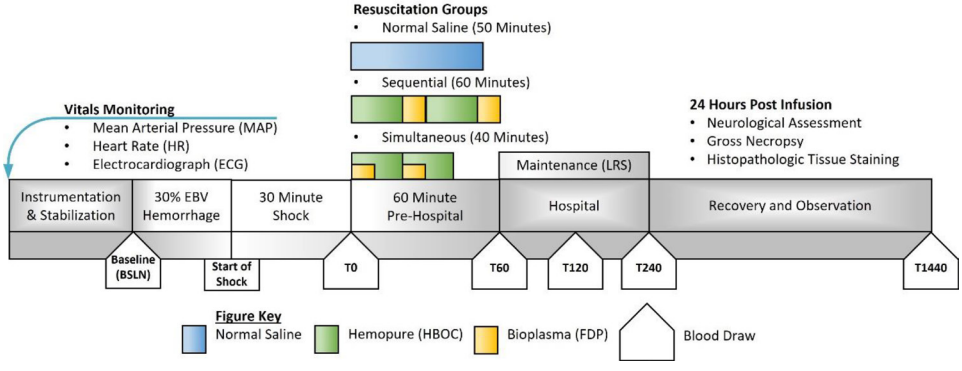

Figure 1 Experimental timeline. T0–T1440 depict time in minutes after the 30-minute shock period. EBV, estimated blood volume; FDP, freeze-dried plasma; HBOC, hemoglobin-based oxygen carrier; LRS, lactated Ringer's solution.

After 30 minutes of shock (T0), experimental treatments were administered (resuscitation phase; figure 1). Doses were calculated based on the equivalent human dose using 70 mL blood/kg as the estimated human blood volume.[22] NHP size unit equivalent volumes were calculated based on animal body weight (online supplemental tables 1 and 2).

NS treatment group: the standard prehospital crystalloid resuscitation in South Africa is up to 1000 mL, in boluses of 200 mL (one-fifth of total), each during 5–10 minutes, with reassessment based on hemodynamic response.[23 24] Total volume of NS administered to NHPs was equivalent to 20.4% EBV (online supplemental tables 1 and 2).

Hemopure and Bioplasma FDP groups (HBOC+FDP): a unit of Hemopure is 250 mL. A unit of reconstituted Bioplasma FDP is 200 mL. Bioplasma FDP was reconstituted according to manufacturer's specifications. The recommended rate of administration for Hemopure is 1 unit/25 min but can be adjusted.[15] This study used 1 unit/20 min. The recommended rate of infusion for Bioplasma FDP is <1 mL/kg/min (Bioplasma FDP package insert). We used a rate of 1 unit Bioplasma FDP/10 min. Each animal received two NHP size units of Bioplasma FDP and two of Hemopure (online supplemental tables 1 and 2).

The resultant total infusion time for NS was 50 minutes. The time for the HBOC+FDP sequential administration group was 60 minutes (20+10+20+10). The total time for the HBOC+FDP simultaneous administration group was 40 minutes ((20 total for FDP and HBOC)×2). For both simultaneous and sequential administration, the products were infused via separate lumens of the triple-lumen infusion catheter, with a saline flush after each unit. A second unit of Bioplasma FDP was not initiated until the initiation of the second unit of Hemopure during simultaneous administration. The resuscitation phase and the timing of the post-resuscitation blood sample were standardized to 60 minutes.

Animals remained anesthetized through 240 minutes for continuous physiological monitoring and blood sampling. During this time, animals received maintenance lactated Ringer's solution at a rate of 4 mL/kg/hr for the first 10 kg of animal weight, and 2 mL/kg/hr for each kg above 10 kg body weight. Next, animals were recovered from anesthesia, returned to their cages, and monitored for health and pain control. Buprenorphine sustained release (0.2 mg/kg) was administered subcutaneously 30 minutes prior to cessation of anesthesia to assure that the animals were comfortable through the 24-hour study period. Acute pain was treated with Buprenex (0.01–0.03 mg/kg) via intramuscular injection, as deemed necessary by a veterinarian. Feed and water were continuously available after recovery from anesthesia. Every 4 hours, neurological/cognitive deficit was assessed by veterinary staff (eg, ability to regain respiratory drive, consciousness, alertness, responsiveness to environmental stimuli, regain upright posture, ambulation, and feeding/drinking) using an established checklist (online supplemental table 3). After final neurological assessment and the final blood collection at 24 hours, animals were humanely killed by intravenous administration of pentobarbital (Euthasol, Virbac Corporation, Fort Worth, TX) at a dose greater than 86.7 mg/kg.

Arterial blood samples were collected at baseline, T0, T60, T120, and T240 (figure 1). The 24-hour (T1440) sample was a venous sample collected under anesthesia. At each time point, the following parameters were analyzed. Arterial blood gas parameters (venous sample at T1440) were assessed using a GEM Premier 4000 (Instrumentation Laboratory, Bedford, MA). Complete blood counts were evaluated via ProCyte Dx Hematology Analyzer (IDEXX Laboratories Inc., Westbrook, ME) and serum chemistries (alanine aminotransferase, amylase, aspartate aminotransferase, blood urea nitrogen, calcium, creatinine, glucose, lactate, lipase, phosphorus) were evaluated on a Catalyst One Chemistry analyzer (IDEXX Laboratories Inc., Westbrook, ME). Cardiac troponin (cTRP) was measured using the iStat (Abbott Point of Care, East Princeton, NJ). Citrated whole blood viscoelastic clotting properties were evaluated by thromboelastography using the RapidTEG reagent (Thromboelastograph (TEG); Haemonetics Corporation, Boston, MA). Prothrombin time, activated partial thromboplastin time, fibrinogen and D-dimer were analyzed using an STA-Compact Max Coagulation Analyzer (Parsippany, NJ).

Immediately after euthanasia, necropsy was performed to include gross evaluation of brain, heart, lungs, liver, kidney, spleen, intestines, and skeletal muscle, as well as major blood vessels and vascular organs. Representative sections of brain, heart, lung, kidney, spleen, pancreas, stomach, ileum, and colon were collected for histopathologic evaluation. Tissue sections were fixed, stained with H&E, and examined by a board-certified veterinary pathologist using an Olympus BX51 upright brightfield microscope (Olympus Scientific Solutions, Waltham, MA). Although special staining for fibrinogen and platelets was not performed, microthrombosis was specifically addressed in the histopathologic evaluation.

This study was powered to include six animals in each of three treatment groups: NS infusion, sequential Hemopure and Bioplasma FDP infusion, and simultaneous Hemopure and Bioplasma FDP infusion. Sample size was determined based on the ability to detect an increase in cTRP of 1 µg/L.[25] Assuming an SD of 0.5 µg/L, and with a power of 90% to detect a 1 µg/L difference (two-sided) in cTRP, six animals per group are required.

Due to the COVID-19 pandemic, there was a worldwide shortage of rhesus macaques for research.[26] As a result, we were not able to obtain sufficient animals for the full study and it became necessary to stop the study after the first nine animals. The first three animals were not randomized. These were planned to be method refinement animals (one received sequential and two received simultaneous HBOC and FDP administration). Because only very minor study adjustments during refinement were implemented (not impacting treatment administration or outcome measurement), these animals were included in the results and analyses. Due to the smaller number of animals available than had been planned according to the power analysis, the sequential administration (n=3) and simultaneous administration (n=4) groups were considered a single group of seven for data interpretation (HBOC+FDP group). Two animals received NS.

Statistical analyses were performed using Prism V.9 (GraphPad Software Inc., La Jolla, CA). Data are presented as mean±SD. Significance was defined at $p<0.05$. Comparisons between groups for continuous variables were performed by t-test. Comparisons related to changes over time within the HBOC+FDP group were performed by one-way repeated measures analysis of variance (ANOVA) with Dunnett's test. ANOVA was not performed for the NS group owing to small sample size. Instead, changes over time in the NS group were evaluated by Friedman test with Dunn's correction. Post-hoc power analysis was performed using G*Power V.3.1 Statistical Power Analysis program (Heinrich Heine Universitat Dusseldorf, Dusseldorf, Germany). With n=7 and an effect size of 0.60 µg/L, analysis for cTRP achieved a power of 0.9998 to detect a change from baseline.

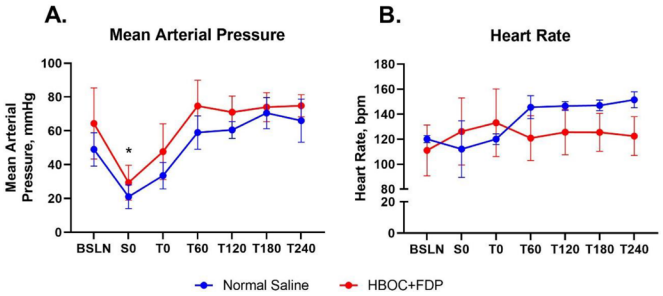

Figure 2 (A) Cardiovascular response to hemorrhage and resuscitation. (B) Heart rate after hemorrhage and resuscitation. Comparisons related to changes over time within the HBOC+FDP group were performed by one-way repeated measures analysis of variance (ANOVA) with Dunnett's test. ANOVA was not performed for the NS group owing to small sample size. Changes over time in the NS group were evaluated by Friedman test with Dunn's correction. *Denotes different from baseline in the HBOC+FDP group ($p<0.05$). bpm, beats per minute; BSLN, baseline; FDP, freeze-dried plasma; HBOC, hemoglobin-based oxygen carrier; NS, normal saline.

RESULTS

There were no significant differences between treatment groups in any cardiovascular or physiologic parameter at any time point. Mean arterial pressure (MAP) declined significantly in response to 30% hemorrhage in the HBOC+FDP group (figure 2A). One animal displayed a hypertensive response to hemorrhage, which occurred prior to resuscitation and was grossly abnormal and different from all other animals (online supplemental figure 1). However, the animal was continued on the study, having met initial inclusion requirements. For characterization of the MAP responses of the NS and HBOC+FDP groups, this animal was excluded (T0 systolic blood pressure >3 SD from the mean). This animal was included for all other analyses. HR increased numerically but not significantly during the shock period (figure 2B).

There was no evidence of a transient spike in blood pressure in any animal. Patterns of sustained blood pressure elevations during infusion were similar between the NS and HBOC+FDP animals (online supplemental figures 2–10). No differences in abnormal heart rhythms were apparent between animals that received NS versus HBOC+FDP (table 1). Arrhythmias were most common during the hemorrhage and shock period (present in four of nine total animals).

There were no significant differences in hematological parameters between treatment groups at any time point (table 2). Red cell counts, hematocrit, and hemoglobin were significantly decreased at T0 and continued to decline through T1440. Platelets significantly declined at T60 through T1440. HBOC+FDP white cell and neutrophil counts were significantly elevated at T1440, and monocyte counts were significantly increased at T240 through T1440, whereas lymphocyte counts were significantly decreased from T60 through T240, but returned to baseline levels by T1440.

No differences between groups were observed at any time point for any clinical chemistry parameter (table 3). HBOC+FDP creatinine significantly declined at T60 through T1440, whereas blood urea nitrogen increased. Serum alanine aminotransferase was significantly elevated at T1440 and aspartate aminotransferase concentrations increased significantly from T60 through T1440. Amylase and lipase were significantly lower from T60 through T240. Phosphorus was elevated transiently from T60 to T120.

No differences were noted between treatment groups at any time point for prothrombin time, activated partial thromboplastin time, fibrinogen, or D-dimer (table 4). HBOC+FDP fibrinogen was significantly lower than baseline from T0 through T120, whereas D-dimer was significantly elevated from T120 and T1440. TEG R, alpha angle, MA, and LY30 were stable throughout the study period, with no differences between treatment groups at any time point (table 4).

All animals recovered normally and exhibited normal 24-hour neurological scores in both NS and HBOC+FDP groups (1.43 ± 1.40 vs. 1.75 ± 1.77, respectively; $p=0.79$).

Postmortem analyses; gross findings: there was no evidence of gross or diffuse hemorrhage in any tissue from any animal in either study group at necropsy. One animal in the NS group had a thrombosis of a large pulmonary artery.

Microscopic findings: there were no differences evident between groups for congestive, inflammatory, and degenerative changes (online supplemental table 4). Inflammatory changes were common. All animals in the study exhibited single-cell necrosis in cardiac tissue sections (may have occurred premortem or may have been a postmortem contraction artifact).

Table 1 Arrhythmias or abnormal electrical activity before, during, and after hemorrhagic shock

		Experimental time period		
Animal	Treatment group	Pre-hemorrhage baseline	Hemorrhage and shock (baseline–T0)	Resuscitation/treatment infusion period (T0–T60)
12050331	NS	Normal	Isolated wide-inverted T waves	Normal
13081571	NS	Normal	Wide-inverted T waves; shortened/absent P waves (26 min string)	Shortened/absent P waves (3 min string)
13052201	HBOC+FDP	Normal	Normal	Normal
12070831	HBOC+FDP	Normal	Normal	Normal
12062631	HBOC+FDP	Normal	Normal	Normal
12090871	HBOC+FDP	Normal	Isolated upright T waves; isolated PVC	Normal
1404155	HBOC+FDP	Normal	Normal	Normal
14042371	HBOC+FDP	Normal	Normal	Normal
32940	HBOC+FDP	Intermittent isolated PACs; few isolated PVCs	Frequent isolated PACs; ectopic P waves; PVCs in sequence	Intermittent isolated PACs; single PVC

FDP, freeze-dried plasma; HBOC, hemoglobin-based oxygen carrier; NS, normal saline; PACs, premature atrial contractions; PVCs, premature ventricular contractions.

Table 2 Hematological response after hemorrhage and resuscitation

		Baseline	T0	T60	T120	T240	T1440
RBC (10^{12}/L)	NS	4.8±0.2	4.3±0.2	4.0±0.1	4.0±0.0	3.9±0.1	3.7±0.3
	HBOC+FDP	4.8±0.4	4.5±0.4**	3.8±0.3**	3.8±0.3**	3.7±0.3**	3.8±0.5**
Hgb (g/L)	NS	115.0±2.8	102.5±0.7	96.5±3.5	98.0±4.2	95.5±3.5	90.0±12.7
	HBOC+FDP	115.8±7.3	107.4±6.9**	103.7±6.7**	102.7±6.4**	100.0±6.5**	95.1±13.6**
Hct (%)	NS	35.6±1.6	31.6±1.1	29.2±1.8	29.7±2.3	28.7±2.0	26.6±4.3*
	HBOC+FDP	35.2±2.3	32.5±1.9**	27.6±1.9**	27.4±1.9**	26.5±1.8**	27.0±3.9**
Platelets (10^9/L)	NS	277.5±19.1	274.0±8.5	242.0±7.1	252.5±4.9	244.0±12.7	214.5±14.8
	HBOC+FDP	297.7±89.5	291.7±91.6	246.9±79.8**	247.0±83.4**	244.9±85.5**	229.6±70.9*
WBC (1000/µL)	NS	12.4±4.0	12.8±1.3	19.6±3.9	20.3±0.1	19.5±0.2	19.0±2.4
	HBOC+FDP	7.9±2.9	8.2±4.1	8.3±4.0	9.3±2.8	8.5±2.5	14.4±2.6**
NEU (1000/µL)	NS	9.1±3.7	9.5±1.1	16.4±2.8	17.6±0.8	16.9±0.6	15.1±2.8
	HBOC+FDP	4.6±2.4	5.2±3.6	6.0±3.4	7.1±2.2	6.2±2.0	10.7±2.7**
LYMPH (1000/µL)	NS	2.5±0.3	2.6±0.3	2.2±0.6	1.7±0.4	1.3±0.3	2.5±0.7
	HBOC+FDP	2.7±1.0	2.4±0.7	1.6±0.6*	1.5±0.5*	1.4±0.5*	2.5±0.8
MONO (1000/µL)	NS	0.6±0.1	0.6±0.1	1.0±0.4	1.0±0.3	1.3±0.1	1.3±0.4
	HBOC+FDP	0.5±0.2	0.5±0.4	0.6±0.4	0.7±0.3	0.8±0.3*	1.0±0.2**

Times represent pre-hemorrhage (−35 min) and times after start of resuscitation in minutes (0–1440 min). Values are mean±SD.
*Different from baseline (p<0.05); **different from baseline (p<0.01).
FDP, freeze-dried plasma; HBOC, hemoglobin-based oxygen carrier; Hct, hematocrit; Hgb, hemoglobin; LYMPH, lymphocyte; MONO, monocyte; NEU, neutrophil; NS, normal saline; RBC, red blood cell; WBC, white blood cell.

Hemorrhage was microscopically observed in a total of six (one animal with two) of the nine total study animals (four of seven in HBOC+FDP; two of two in NS). Renal hemorrhage was most common (three of seven in HBOC+FDP; two of two in NS) and was graded minimal to mild. Brain microhemorrhage (graded mild) was observed in two animals (two of seven in HBOC+FDP; zero of two in NS). The single thrombotic finding (zero of seven in HBOC+FDP; one of two in NS) was a partially occlusive arterial thrombus (graded moderate).

DISCUSSION

We were not able to obtain sufficient animals and had to stop the study after the first nine animals due to a worldwide shortage of rhesus macaques for research.[26] Nonetheless, this is the largest NHP study examining either Hemopure or Bioplasma FDP, and the first study in any species incorporating both products. The safety profiles of the individual products, Hemopure[13–15] and Bioplasma FDP,[7 8] have been reported. The specific question

Table 3 Metabolic and chemistry parameters

		Baseline	T0	T60	T120	T240	T1440
Lactate (mM/L)	NS	1.0±0.3	1.8±1.5	1.6±1.1	1.5±0.8	1.3±0.6	2.2±0.6*
	HBOC+FDP	0.8±0.2	1.1±0.6	1.1±0.2	1.1±0.2	0.9±0.1	2.4±0.9*
Base excess	NS	0.8±2.1	−0.4±0.8	−1.0±0.8	−1.1±1.2	−0.6±0.8	4.6±0.1*
	HBOC+FDP	1.0±3.2	0.1±2.7	2.1±2.7	2.6±2.7	2.7±2.9	4.7±3.4*
GLU (mg/dL)	NS	75.5±2.1	103.5±20.0	75.5±9.2	77.0±12.7	81.5±7.8	113.0±17.0
	HBOC+FDP	70.1±12.7	89.1±23.1*	79.6±7.1	75.9±6.5	78.3±7.5	92.9±25.7
CREA (mg/dL)	NS	1.1±0.1	1.4±0.2	1.1±0.2	1.0±0.1	0.8±0.1	0.7±0.1
	HBOC+FDP	1.0±0.1	1.1±0.2	0.7±0.2**	0.7±0.1**	0.6±0.2**	0.6±0.1**
BUN (mg/dL)	NS	12.5±0.7	14.0±1.4	14.5±0.7	16.0±2.8	17.5±2.1	28.5±6.4*
	HBOC+FDP	15.0±6.9	16.1±6.8**	18.6±7.1**	19.7±6.5**	21.6±6.2**	23.3±5.7
PHOS (mg/dL)	NS	6.1±0.1	7.5±1.1	7.4±0.7	7.0±0.5	6.0±0.2	5.2±1.5
	HBOC+FDP	6.0±1.0	6.5±1.1	7.8±1.0**	7.2±0.8**	6.5±0.8	6.7±4.2
Ca (mg/dL)	NS	8.6±0.0	8.3±0.0	7.5±0.4	7.7±0.6	7.8±0.2	8.1±0.6
	HBOC+FDP	8.5±0.4	8.3±0.5	8.4±0.4	8.3±0.5	8.1±0.5	8.7±0.6
ALT (units/L)	NS	30.0±1.4	25.5±5.0	31.5±2.1	32.5±9.2	32.0±0.0	734.0±323.8
	HBOC+FDP	37.0±16.3	37.4±16.0	50.1±9.2	55.4±11.5	51.0±13.5	362.3±227.4*
AST (units/L)	NS	20.0±2.8	19.0±2.8	20.0±5.7	20.5±0.7	28.5±7.8	2013.0±1315.2
	HBOC+FDP	24.3±4.5	23.4±6.5	31.6±7.1*	36.4±10.4*	58.9±21.2*	1049.7±584.6*
AMYL (units/L)	NS	343.5±36.1	327.5±44.6	279.5±58.7	293.0±28.3	278.5±27.6	354.5±43.1
	HBOC+FDP	380.7±52.8	367.6±52.6	334.6±46.6**	319.4±58.3**	323.6±45.0**	653.3±258.7
LIPA (units/L)	NS	111.5±48.8	109.5±30.4	92.0±15.6	92.5±14.8	86.5±26.2	210.0±113.1
	HBOC+FDP	80.3±20.2	83.4±18.6	36.4±17.9**	42.7±12.7**	50.0±19.1*	255.3±207.2
cTRP (ng/mL)	NS	0.05±0.06	0.07±0.08	0.07±0.10	0.07±0.08	0.05±0.05	2.06±2.54
	HBOC+FDP	0.01±0.01	0.00±0.00	0.00±0.00	0.01±0.01	0.03±0.07	0.46±0.73

Times represent pre-hemorrhage (−35) and times after start of resuscitation in minutes (0–1440 min).
*Different from baseline (p<0.05); **different from baseline (p<0.01).
ALT, alanine aminotransferase; AMYL, amylase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Ca, calcium; CREA, creatinine; cTRP, cardiac troponin; FDP, freeze-dried plasma; GLU, glucose; HBOC, hemoglobin-based oxygen carrier; LIPA, lipase; NS, normal saline; PHOS, phosphorus.

Table 4 Plasma and whole blood coagulation parameters

Parameter		Baseline	T0	T60	T120	T240	T1440
PT (s)	NS	13.4±0.4	13.9±0.2	14.3±0.3	15.4±2.2	14.5±0.5	13.3±0.1
	HBOC+FDP	14.2±0.9	14.3±0.9	14.9±0.9	15.7±2.1	14.9±1.2	13.3±1.2
aPTT (s)	NS	26.5±1.1	25.6±3.0	25.4±2.8	27.6±6.5	26.5±2.5	29.5±1.8
	HBOC+FDP	26.3±1.9	25.7±2.0	26.6±1.6	26.9±3.4	25.5±1.5	27.3±2.2
Fibrinogen (mg/dL)	NS	247.0±75.0	223.5±61.5	209.0±46.7	192.0±77.8	201.5±64.3	391.5±31.8
	HBOC+FDP	195.3±29.0	182.6±29.0*	172.7±26.8**	158.6±29.7*	170.6±24.1	311.9±128.6
D-dimer (µg/mL)	NS	0.8±0.5	0.6±0.0	0.6±0.1	1.0±0.9	1.3±0.5	1.9±0.1
	HBOC+FDP	1.0±0.6	0.9±0.5	1.2±0.4	1.5±0.5*	1.4±0.7	1.9±0.6*
R (min)	NS	0.7±0.2	0.8±0.1	0.7±0.4	0.7±0.4	0.7±0.2	0.6±0.2
	HBOC+FDP	0.7±0.1	0.8±0.1	0.8±0.2	0.7±0.3	0.6±0.3	0.7±0.1
Alpha-angle (degrees)	NS	78.8±0.9	77.7±0.8	77.4±1.8	80.2±1.8	78.4±2.0	79.3±0.7
	HBOC+FDP	74.6±6.3	76.8±2.5	77.1±2.6	76.2±1.8	76.2±3.1	76.2±8.4
MA (mm)	NS	74.1±0.9	73.1±1.8	70.0±10.7	73.3±10.7	72.7±8.2	78.7±4.2
	HBOC+FDP	62.1±6.4	65.0±6.9	64.2±6.0	60.1±14.3	65.7±6.6	66.7±23.8
LY30 (%)	NS	0.3±0.4	0.4±0.5	0.1±0.1	0.1±0.1	0.0±0.0	0.4
	HBOC+FDP	1.4±1.8	0.6±0.6	0.4±0.5	0.7±0.8	0.2±0.3	0.3±0.3

*Different from baseline (p<0.05); **different from baseline (p<0.01).
aPTT, activated partial thromboplastin time; FDP, freeze-dried plasma; HBOC, hemoglobin-based oxygen carrier; NS, normal saline; PT, prothrombin time.

for the present study was whether the use of the two products together presents a safety risk.

As expected after severe hemorrhage, blood pressure declined significantly across treatment groups.[19] Numeric patterns after resuscitation were suggestive of an improved cardiovascular response in the HBOC+FDP group, but this was not confirmed statistically. This is to be expected as crystalloid solutions have substantially shorter intravascular retention times than colloid preparations.[27] We examined several parameters to comprehensively assess cardiac safety. None of these indicated any differences between groups. To ensure that we did not miss any brief spikes in blood pressure, we examined minute-by-minute blood pressure (online supplemental figures 2-10). Individual blood pressure responses during the resuscitation period showed no acute spikes and no individual sustained responses suggestive of a difference in safety response between the two treatment groups. Even in one animal that started the resuscitation period with a pre-existing hypertension (systolic blood pressure approximately 160 mm Hg; online supplemental figure 8), there was no additional hypertensive response associated with the HBOC+FDP infusion.

To address concerns about potential cardiac adverse events related to Hemopure,[14] the present study was originally powered to detect an elevation in cTRP and included a comprehensive evaluation of cardiac parameters. Serum cTRP concentrations did not differ between groups at any time point (table 3). No differences in ECG responses to hemorrhage and resuscitation were apparent between treatments (table 1). During the resuscitation period, one animal in each of the NS and HBOC+FDP groups exhibited arrhythmias, which appeared to be improved, compared with the hemorrhage and shock period in each respective animal. Histopathology revealed single-cell necrosis in cardiac tissue from all animals. Cardiac single-cell necrosis has previously been observed in an ovine model of hemorrhagic shock.[28] Regardless of causation, there was no difference between treatment groups in this finding.

A 2008 meta-analysis that bundled data from numerous HBOC formulations concluded that HBOCs as a class were associated with increased risk of cardiac injury.[29] However, when specifically examining Hemopure, no increased risk of cardiac injury has been observed. Levien reviewed[13] hemovigilance data from 336 patients in South Africa who received Hemopure, and found that patients responded well clinically with no pattern of significant Hemopure-attributable significant adverse events. Only 5% of patients experienced a transient elevation in systolic blood pressure greater than 30 mm Hg, and all resolved either by reducing the infusion rate or treatment with standard medications. Mackenzie *et al* reviewed data from 1700 patients who received Hemopure and found no increase in adverse events related to the product.[14] In the present animal study, the comprehensive set of blood pressure and cardiac parameters examined suggests no evidence of cardiac risk.

There were no differences between treatment groups in any specific organ or metabolic parameter examined. Histopathologic findings of vascular congestion and inflammatory changes in lung tissue were common and similar between groups. Pulmonary vascular and inflammatory changes are well-known responses to hemorrhagic shock in both patients[30 31] and in experimental models.[28 32 33] Serum creatinine and blood urea nitrogen increased similarly in both treatment groups by 1440 minutes (24 hours) after hemorrhage. Renal vascular congestive, inflammatory, and degenerative findings were similar for the two groups. Acute kidney injury is associated with hemorrhagic shock in patients[34] and has been well documented in animal models of hemorrhagic shock.[28 35 36] Consistent with the renal response to severe hemorrhage, liver enzymes were elevated by 24 hours post-hemorrhage in both groups, coincident with hepatic degenerative/necrotic changes. Similar observations have recently been reported after severe hemorrhage in sheep.[28] Pancreatic enzymes were numerically higher at 24 hours, also with no difference between groups. Data indicate pulmonary, renal, hepatic and pancreatic injury, which was similar between groups, and was consistent with expectations for severe hemorrhagic shock.

Hemostasis and thrombosis-related parameters were similar between treatment groups. Changes in coagulation parameters in both groups over time suggest that hemorrhagic shock induced a slightly hypercoagulable state, as has been observed previously in NHP.[19] There were no changes in viscoelastic clotting parameters in either group during the study period, suggesting normal overall whole blood hemostatic and fibrinolytic function for

both groups. The lack of altered coagulation parameters in this study after shock may have been because the animals experienced isolated hemorrhage without polytrauma. The combination of shock and tissue injury drives trauma-induced coagulopathy in human patients,[37] and this combination more reliably produces a detectable coagulopathy in animal models than either shock or injury alone.[38] This severe hemorrhagic shock model was associated with thrombotic and/or hemorrhagic findings in most animals. The thrombosis observed in one NS animal may have been a result of a hypercoagulable response to NS resuscitation, which has been observed both in vitro and in vivo in humans.[39 40] Five microscopic hemorrhages were renal and these occurred in both groups. Renal hemorrhages have previously been reported after hemorrhagic shock in rats.[35] The observed microhemorrhages in brain tissues were also likely a result of the severe hemorrhagic shock produced in this NHP model, as these have previously been reported after hemorrhagic shock in a sheep model.[28] There have been no previous reports of brain microhemorrhages associated with either Hemopure or Bioplasma FDP. There is no a priori reason to expect a coagulopathy associated with combining these therapeutics.[41]

In the present study, there was no neurological deficit or difference in recovery in the animals with the observed brain microhemorrhages. The potential clinical implications of this finding are unclear, since brain microhemorrhages can be asymptomatic and the prevalence in normal adults is 5%.[42] There has been an association with hypertension and old age.[42–44] None of the animals in the study experienced a spike in blood pressure during the infusion period. One of the two animals (online supplemental figure 7) appeared to have had a reduced cardiovascular compensatory response during the pre-infusion shock period (pre-resuscitation MAP <20 mm Hg), which potentially contributed to the histopathologic findings.

An important limitation is that we were not able to obtain sufficient animals for the full study, limiting the power to make statistical comparisons. The study was originally planned for six animals per treatment group. By combining the animals that received sequential and simultaneous patterns of HBOC+FDP administration, the characterization of the HBOC+FDP group is based on seven animals. Therefore, as this number is in accord with our a priori power analysis, we think that the study provides a credible safety evaluation.

CONCLUSION

Overall findings indicate that there was no evidence of harm associated with the combined use of Hemopure and Bioplasma FDP for resuscitation from hemorrhagic shock. We observed a similar safety profile for NS versus HBOC+FDP when administered as resuscitative treatments after severe hemorrhagic shock in NHP. No differences were noted in safety-related cardiovascular, pulmonary, renal or other organ or metabolic parameters between animals in the NS versus HBOC+FDP treatment groups. Findings for hematological, hemostasis, and thrombosis-related parameters were similar between the two groups, and where numeric (non-statistical) differences were observed, these were consistent with well-established responses to hemorrhagic shock as reported in the literature for animal models in other species. No differences were noted in neurological findings or overall survival and recovery. Findings of this NHP study are supportive of a potential clinical study of the use of Hemopure and Bioplasma FDP to treat patients with hemorrhagic shock in the out-of-hospital setting.

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