Hematology patterns after hemoglobin-based oxygen carrier resuscitation from severe controlled hemorrhage with prolonged delayed definitive care

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BACKGROUND: The hemoglobin-based oxygen carrier (HBOC-201) resuscitation fluid improves outcome in hemorrhagic shock swine models with minimal coagulopathy. Herein, coagulation parameters were evaluated after resuscitation with HBOC-201 after severe bleeding and prolonged delay to definitive care.

STUDY DESIGN AND METHODS: After 55 percent estimated blood volume–controlled hemorrhage by catheter withdrawal, swine (n = 48) were resuscitated with HBOC-201 or Hextend (HEX) infused in four doses over 4 hours or not resuscitated (NON). Animals were randomly assigned in two cohorts of 4- or 24-hour simulated delay to hospital arrival (access to blood and saline infusions up to 72 hr). In vitro hematologic monitoring was assessed with complete blood count, hemostasis (thromboelastography [TEG], in vitro bleeding time [PFA]), and coagulation (prothrombin time [PT], thrombin-antithrombin, fibrinogen) indices.

RESULTS: Within groups, survival was unaffected by extending delay from 4 to 24 hours. Combined survival was similar for HBOC-201 and HEX but lower for NON animals (93.5, 81.5, and 25 percent, respectively; $p < 0.01$). Blood transfusion requirements were lower with HBOC-201 than HEX. Elevated TEG and PFA parameters in resuscitated animals reflected fluid and blood transfusion regimens. TEG reaction time and PFA were transiently higher with HBOC-201 than with HEX during the early hospital phase. PT was increased in HEX animals.

CONCLUSION: In this severe model, survival was equivalent with HBOC-201 and HEX resuscitation. HBOC-201 or HEX allowed delayed hospital arrival to 24 hours without worsening coagulation parameters, but dilutional mild coagulopathy in the hospital phase persisted with HBOC-201 due to blood transfusion avoidance. Low hematocrit suggests that blood administration after HBOC-201 resuscitation could be beneficial to replete blood cellular mass.

Mortality from severe hemorrhage in the hour after injury can be reduced by early intervention.[1,2] Nonetheless, development of coagulopathy during trauma and subsequent resuscitation is common after the traumatic insult and infusion of large volumes of fluid.[3] Both platelets (PLTs) and coagulation factors, initially consumed at the wound site, are further reduced after fluid resuscitation. This disruption of the hemostasis balance may lead to disastrous pathophysiologic consequences including

ABBREVIATIONS: AT-III = antithrombin-III; EBV = estimated blood volume; HBOC = hemoglobin-based oxygen carrier; HEX = Hextend; ICU = intensive care unit; MAP = mean arterial pressure; NON = no fluid resuscitation; PFA = in vitro bleeding time; PT = prothrombin time; TAT = thrombin-antithrombin; TEG = thromboelastography; TEG-CI = TEG coagulation index; TEG-MA = TEG maximum amplitude; TEG-R = TEG reaction time.

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disseminated intravascular coagulopathy and multiorgan failure (MOF) in severe trauma patients.[4] New resuscitation low-volume fluids, such as hemoglobin-based oxygen carrier-201 (HBOC-201, Biopure Corp., Cambridge, MA), stabilize hemodynamics and tissue oxygenation in moderately hemorrhaged swine.[5] In addition, in severe hemorrhagic shock, HBOC-201 decreases blood lactate and increases survival.[6-8] HBOC-201's capability to supply oxygen to tissues and organs may allow safe transportation of wounded to centers where definitive care is available despite significant prehospital delay.

The hemostatic effects of resuscitation fluids were compared in previous studies simulating a 4-hour delay to hospital arrival in both moderately severe (40% estimated blood volume [EBV]-controlled hemorrhage) and severe uncontrolled hemorrhage (liver injury).[9,10] These studies showed that HBOC-201 resuscitation resulted in less coagulopathy than Hextend, a buffered hydroxyethyl starch solution (HEX, Hextend, Abbott Laboratories, Abbott Park, IL) during the prehospital phase due to less hemodilution, but more coagulopathy during the hospital phase as blood transfusion requirements were lower. Coagulation patterns after HBOC-201 resuscitation in severe hemorrhage models simulating longer delay to definitive care have not been reported.

In military contingencies, rural or austere environments, and extreme sports, low-volume resuscitation resulting in good clinical outcome despite significant delay to hospital care may be a distinct advantage and a powerful tool for emergency medicine providers. A 24-hour delay could potentially influence overnight evacuation or evacuation in difficult environments. This study examined whether the mild coagulopathic effects of HBOC-201 observed with moderate traumatic hemorrhage would persist in more severe conditions of blood loss and delayed evacuation.

MATERIALS AND METHODS

These experiments were conducted according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. The study was approved by the Walter Reed Army Institute of Research and Naval Medical Research Center Institutional Animal Care and Use Committee. All procedures were performed in an animal facility accredited by the American Association for Accreditation for Laboratory Animal Care.

Animal procedures

Animal and hemorrhage model. This model has been described previously.[11] Swine were used in a model simulating severe traumatic hemorrhagic shock caused by 55 percent blood loss–controlled hemorrhage. Yucatan minipigs (n = 48) were anesthetized (ketamine-isofluorane induction and isofluorane maintenance), intubated, and allowed to breathe spontaneously (FiO_2, 21%). Rectal temperature was monitored and maintained at 37 to 38.2°C with a warming device (Model 505, Bair Hugger, Augustine Medical, Eden Prairie, MN). The external jugular vein and carotid artery were catheterized by open technique for vascular access and continuous blood pressure monitoring. A pulmonary artery catheter was inserted. After an equilibration period (5-10 min), animals were hemorrhaged to 55 percent of their EBV by catheter withdrawal over a 15-minute period. To mimic soft tissue injury, the rectus abdominus muscle was crushed in a standardized fashion for 5 minutes with a Kocher clamp at Time 0, concomitant to the start of blood shed by catheter withdrawal. Phlebotomy blood volumes (totaling approx. 95 mL or 4% EBV for animals surviving 240 min) were not included in reported hemorrhage volumes. To simulate battlefield delay, the animals were left in shock for an additional 5 minutes after the end of blood withdrawal, at which time (Time 20 min) they received the first resuscitation fluid infusion at 10 mL per kg over 10 minutes. Subsequently, animals were resuscitated with 5 mL per kg fluid over 10 minutes at 30, 60, 120, and 180 minutes if prospectively defined criteria were met (mean arterial pressure [MAP], < 60 mmHg; or heart rate, > baseline). Animals were intensively monitored for 4 hours during this prehospital phase but received only fluid resuscitation. After 4 hours, carotid and pulmonary arterial catheters were removed, and neck and abdominal skin and fascia were closed. The animals were then recovered from anesthesia and divided into two cohorts for this phase: a 4-hour delay cohort in which animals received immediate hospital care, simulated by surgical repair and availability of blood transfusions and additional crystalloid fluid (saline) at 4, 24, and 48 hours after injury; and a 24-hour delay cohort in which animals received the same hospital care treatment at 24 and 48 hours after injury. Whole-blood transfusions were administered for Hb levels of less than 7 g per dL (in accordance with recent guidelines)[12] and saline for Hb levels of more than 7 g per dL at 10 mL per kg. Animals were euthanized at 72 hours. Shed blood from the animal was collected in blood bags containing standard anticoagulant (CPD-A, Fenwal, Deerfield, IL). The blood was used for autologous transfusion or was stored for potential use in allogenic transfusions for other animals during the hospital phase.

Fluid resuscitation. At Time 20 min (end of the shock phase), animals were randomly allocated to one of three resuscitation study groups, receiving HBOC-201 (n = 8), buffered HEX (n = 8), or no fluid resuscitation (NON; n = 8). HBOC-201 is purified, filtered, stroma-free, and heat-treated bovine Hb and polymerized by gluteraldehyde crosslinking to form polymers ranging from 130- to

500-kDa molecular weight. HBOC-201 is prepared in a buffer similar to lactated Ringer's solution containing a 50:50 racemic D- and L-lactate mixture (27 mEq lactate), *N*-acetyl-polycysteine (0.17%), approximately 12.5 g Hb per dL, with an oncotic pressure of 17 mmHg, an osmolality of approximately 300 mOsmol per kg, a pH value of approximately 7.8, and an oxygen affinity (P_{50}) of 38 mmHg (lower than that of human blood). HBOC-201 does not contain glucose and is stable at 25°C for more than 3 years. HEX is 6 percent hydroxyethyl starch (molecular weight, 670 kDa) prepared in balanced lactated Ringer's solution (50:50 racemic mixture, 28 mEq lactate), containing glucose (1 g/L), with a pH value of approximately 6.6, an osmolality of 307 mOsmol per kg, and an oncotic pressure of 30 mmHg (Hextend, Abbott Laboratories). HEX has been recommended as the standard resuscitation fluid for US Special Forces for battlefield care.[13]

Fluid infusion was computed as the volume of fluid infused per kilogram per surviving animal (each infusion was 10 mL/kg) during the prehospital phase and similarly for the number of blood transfusions or saline infusions during the hospital phase. For each treatment group, the number of infusions was summed for all animals at each time point and then divided by the number of animals surviving at this time. For example, if all eight animals survived the entire 240 minutes, the maximum allowable volume per kilogram infused at 20, 30, 60, 120, and 180 minutes would have been 30 mL per kg, and the maximum allowable volume of blood transfused or saline infused at 4, 24, and 48 hours would have been 30 mL per kg. This was then cumulated at each time point until the animal died or reached the end point. This calculation eliminates the confounding of final fluid infusion volumes resulting from animal death. Also, these normalized data could be compared to a maximum number of theoretical infusions and then further compared to each treatment group.

In vitro assays

Assays.[10] All functional laboratory assays were performed at 37°C, consistent with recorded normothermic animal temperatures (37.5 ± 0.9°C). Thrombosis and hemostasis were assessed as previously described. The following tests were carried out from blood samples collected at 0, 30, 60, 180, and 240 minutes and 24, 48, and 72 hours (in Vacutainer tubes, BD, Palo Alto, CA). Complete blood count with differential was performed with a cell counter (Pentra 60C$^+$, Horiba ABX Diagnostics, Irvine, CA). Plasma hemoglobin (Hb; due to HBOC-201) was measured on a blood gas instrument (ABL 750, Radiometer, Copenhagen, Denmark).

Thromboelastography (TEG) reaction time (TEG-R, corresponding to fibrin formation), kinetics of clot formation (TEG-K and TEG-α), maximum amplitude (TEG-MA), and fibrinolysis (TEG-Ly) were measured with a hemostasis analyzer (TEG 5000, Haemoscope Corp., Niles, IL). The coagulation index (TEG-CI) was calculated as

$$\text{TEG-CI} = (0.0184 \times \text{TEG-K}) + (0.1655 \times \text{TEG-MA}) - (0.0241 \times \text{TEG-}\alpha) - (0.2454 \times \text{TEG-R}) - 5.022.$$

The test was initiated with 340 µL of whole blood recalcified with 20 µL of $CaCl_2$. In vitro bleeding time (PFA) was measured by the closure time of an ADP-collagen–coated capillary after aspiration of 800 µL citrated whole blood with a PLT function analyzer (PFA-100, Dade Behring, Deerfield, IL).

Coagulation parameters, including prothrombin time (PT), fibrinogen, thrombin-antithrombin (TAT), and antithrombin-III (AT-III), were measured with both clot-based principles and colorimetric determination on a coagulation workstation (STA Compact, Diagnostica Stago, Parsippany, NJ). Thrombin-antithrombin (TAT) was determined by enzyme-linked immunosorbent assay (Enzygnost, Dade Behring, FL), and results are expressed as nanograms per milliliter; HBOC-201 did not interfere with this assay. AT-III was not determined for samples containing HBOC-201 because HBOC-201 interferes with the test. There was no significant interference due to plasma HBOC-201 for any of the other reported assays.

Data analysis and statistics

Animals were randomly allocated to experimental groups at 10 minutes into the experiment via envelopes prepared by an outside statistician. Results, data, and figures are presented as means ± standard deviation unless otherwise stated. Prehospital data in the first 4 hours were combined for the 4- and 24-hours delay cohorts because this period was essentially the same for each group (n = 48). Subsequently, the 4- and 24-hour delay cohorts (n = 24 each) were compared. Survival rates were analyzed with Fisher's exact test. For multiple variables and for data collected over time, results were analyzed with the mixed statistical model for global inspection of continuous measurements (Proc Mixed, SAS, Cary, NC). Significant group and time effects were indicated, and when appropriate, individual measures were subsequently compared with a two-tailed paired t test assuming equal variance. A p value of 0.05 was considered significant. Surface under the curve tests were also performed when applicable.

RESULTS

In vivo observations

Overall, the mean weight for all 48 pigs was 35.2 ± 16.5 kg, similar in all groups (Fisher's exact test).

Survival. HBOC-201 and HEX animals had a higher chance of surviving (>90%; NS) to the hospital phase in comparison with NON animals ($p < 0.01$, Fisher's exact test; Table 1). In the hospital phase, survival rates in the two resuscitated groups remained similar, with no difference between the 4- and 24-hour delay cohorts and were higher than in the NON group ($p < 0.01$). Survival rates at the end of the prehospital phase were comparable to those at end of hospital for the respective treatment groups.

TABLE 1. Survival after 55 percent EBV hemorrhage in 4- and 24-hour evacuation delay cohorts

Resuscitation fluids	Delay group (hours)	Prehospital survival (%) 4 and 24 hour cohorts combined	Hospital survival (%)	Hospital survival (%) 4 and 24 hour cohorts combined
HBOC-201	4	100	100	93.5
	24		87.5	
HEX	4	93.8	75.0	81.7
	24		87.5	
NON	4	31.3	25.0*	25.0
	24			

* Fisher exact: $p < 0.01$ between treated and nontreated animals. There was no difference between the prehospital and hospital phases in each treatment group.

Fluid requirement during the first 4 hours of the prehospital phase. Data were combined for fluid requirement in the prehospital phase of the 4- and 24-hour delay cohorts (Fig. 1). There was no difference at 180 minutes between the volume of HBOC-201 infused (27 mL/kg) compared to that of HEX (29 mL/kg) and/or the maximum volume infused (30 mL/kg) for all surviving animals. All animals met fluid requirements (i.e., MAP or heart rate) in both treatment groups for the first three infusions. There was a trend for a lower HBOC-201 infusion requirement ($p = 0.07$) for the last infusion time point during the prehospital phase compared to the maximum allowance (at 180 min, HBOC-201 12/16 vs. HEX 15/16), probably due to an increased MAP.[11]

In vitro HBOC-201 concentration. The fluid resuscitation regimen was identical in both delay cohort as illustrated in Fig. 2, and consequently, the plasma HBOC-201 concentration was similar in both 4- and 24-hour delay groups. The $t_{1/2}$ was calculated to be 19 ± 1.2 hours.

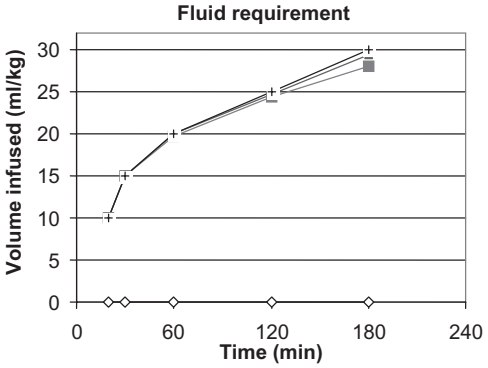

Fig. 1. Fluid requirement after 55 percent controlled hemorrhage. Infusion was computed during resuscitation during the first 4 hours in the prehospital phase as a mean volume infused per surviving animal. The volume was cumulated over time up to 180 minutes (■, HBOC-201; ▲, HEX; ◇, NON; and +, volume for maximal infusion requirement).

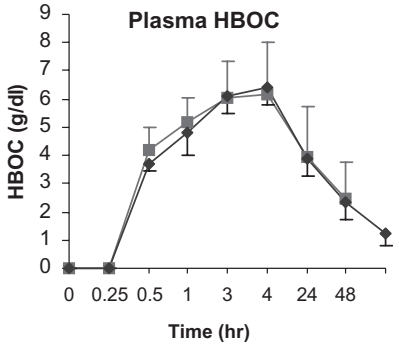

Fig. 2. Plasma Hb resulting from HBOC-201 infusions in blood samples collected during the course of resuscitation after 55 percent EBV hemorrhage in (■) 4- and (◆) 24-hour evacuation delay cohorts.

Hospital phase

Blood transfusion and saline transfusion. Blood and saline volumes infused during the hospital phase were each cumulated over 48 hours for animals surviving to that time (Fig. 3). Animals in the HEX and NON groups required, on average, more blood transfusion than HBOC-201 for the 4- and 24-hour evacuation delays. Evacuation after 24 hours did not significantly change the number of transfusions for HBOC-201 because only one of eight animals required blood in both delay cohorts at 24 hours, whereas all HEX animals required a transfusion at either 4 or 24 hours, depending on the cohort. HBOC-201 and HEX animals had similarly low requirements for saline infusion compared to the maximum requirement ($p < 0.05$). One of two NON animals required saline infusions in the 4-hour delay cohort whereas none required any infusions in the 24-hour delay cohort. This suggested that, in the NON group, animals surviving 4 hours initiated compensation naturally and required few additional blood or saline infusions.

Hematocrit (Hct) level, Hb level, PLT count, and white blood cell (WBC) count (hematology indices) are

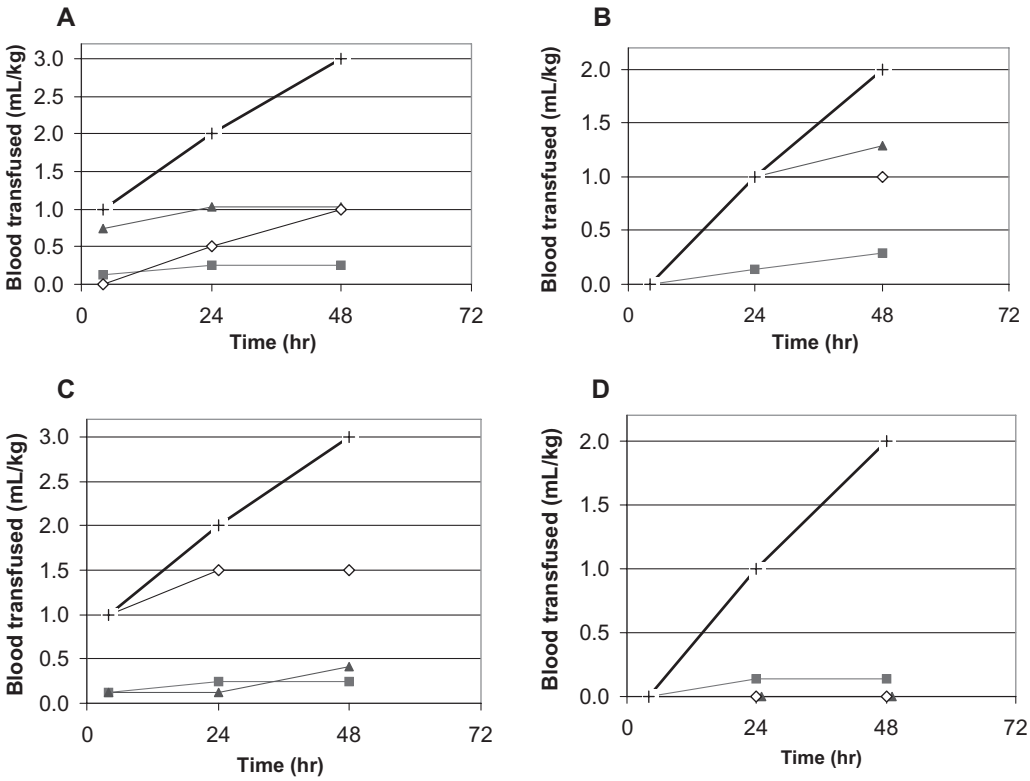

Fig. 3. Blood and saline requirements in the hospital phase after 55 percent controlled hemorrhage. Blood and saline volumes were computed as volume of blood or saline (ml/kg) infused per surviving animal for all treatment groups (■, HBOC-201; ▲, HEX; ◇, NON; and +, maximal infusion requirement). HBOC-201 required fewer blood transfusions ($p < 0.05$). (A) Blood transfusion 4-hour delay; (B) blood transfusion 24-hour delay; (C) saline infusion 4-hour delay; (D) saline infusion 24-hour delay.

shown in Fig. 4A. Hct initially increased from T0 to T15 after injury in all groups (27.9 ± 2.9% vs. 35.0 ± 7.8%; $p < 0.001$) and then diminished in both treatment groups due to hemodilution during the prehospital phase; Hct remained elevated in the NON group. During the hospital phase, Hct was similar in both the 4- and 24-hour cohorts after HBOC-201 resuscitation, but was higher in the 4-hour cohort compared to the 24-hour cohort after HEX resuscitation due to blood transfusions ($p < 0.02$). Hct decreased in NON animals at 4 hours due to saline infusions. Hb as Hct increased at T15 and was higher in NON animals, decreased in HEX animals, and was maintained to baseline in HBOC-201 animals during the prehospital phase. PLT decreased similarly in both resuscitated groups during the prehospital phase (time difference; $p < 0.05$) and were higher in the 4-hour cohort compared to the 24-hour cohort in HEX and HBOC-201 animals ($p < 0.05$). Notably, PLT count decreased in NON animals despite the increased Hct in the prehospital phase. WBC counts were significantly reduced at 15 minutes in all groups ($p < 0.01$) and were higher in NON than HBOC-201 and HEX animals during the prehospital phase due to absence of hemodilution by resuscitation fluids; there were no group differences in WBC counts in the hospital phase. Neutrophils followed the same pattern (data not shown).

Functional indices, including TEG-R, TEG-MA, PFA, PT, fibrinogen, TAT, and AT-III (hemostasis), are shown in Fig. 4B. There were no group differences in TEG-R during the prehospital phase, but TEG-R was significantly increased in HBOC-201 animals during the hospital phase at 24 hours ($p < 0.01$); TEG-K, TEG-α, and TEG-CI essentially followed the same pattern. Reflecting PLT number and function, TEG-MA was lower and PFA higher in both resuscitation groups than in the NON group due to reduced PLT ($p < 0.02$). In the hospital phase, TEG-MA was similar in all groups whereas PFA remained elevated until 24 hours in HBOC-201 animals versus 4 hours for HEX and NON animals (due to delayed blood transfusions). Extension of the prehospital delay from 4 to 24 hours had no effect on any of these parameters in all groups.

Fibrinogen was lower at 4 hours in the resuscitated groups than in the NON group during the prehospital phase, due to hemodilution ($p < 0.02$); being an acute-phase reactant, expectedly, fibrinogen increased ($p < 0.01$) in the hospital phase similarly in all groups. TAT was similar between groups and remained low in all

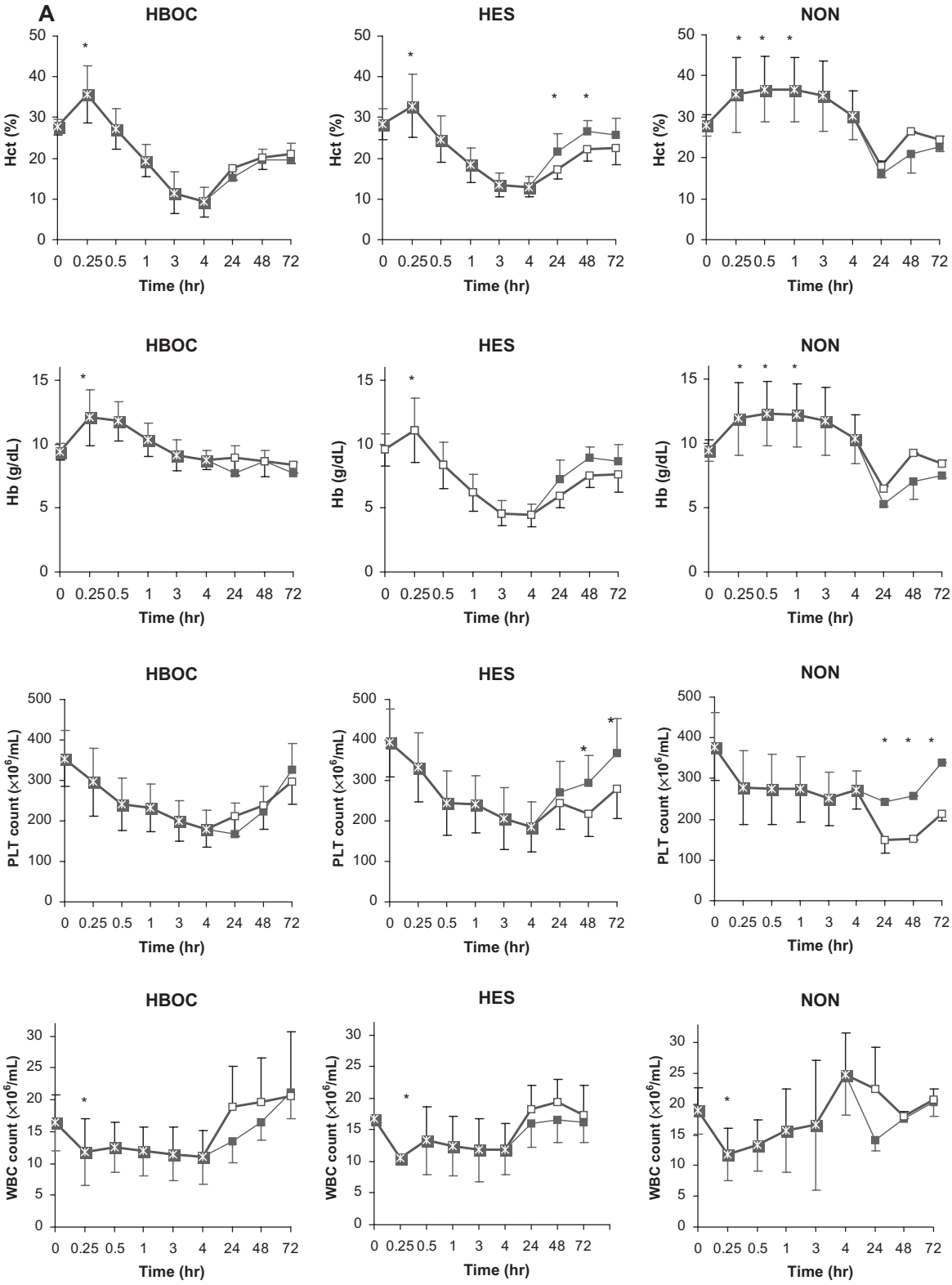

Fig. 4. (A) Hematology and (B) hemostasis parameters after 55 percent controlled hemorrhage in 4- and 24-hour delay cohorts for HBOC-201, HEX, and NON treatment groups. Values are presented as means ± SD. The differences between 4- and 24-hour delay cohorts in the hospital phase for all groups were attributed to blood and saline infusion regimens. (■) 4-hour delay cohort; (□) 24-hour delay cohort; (—⊠—) combined 4- and 24-hour delay cohort. *Significant interactions, $p < 0.05$.

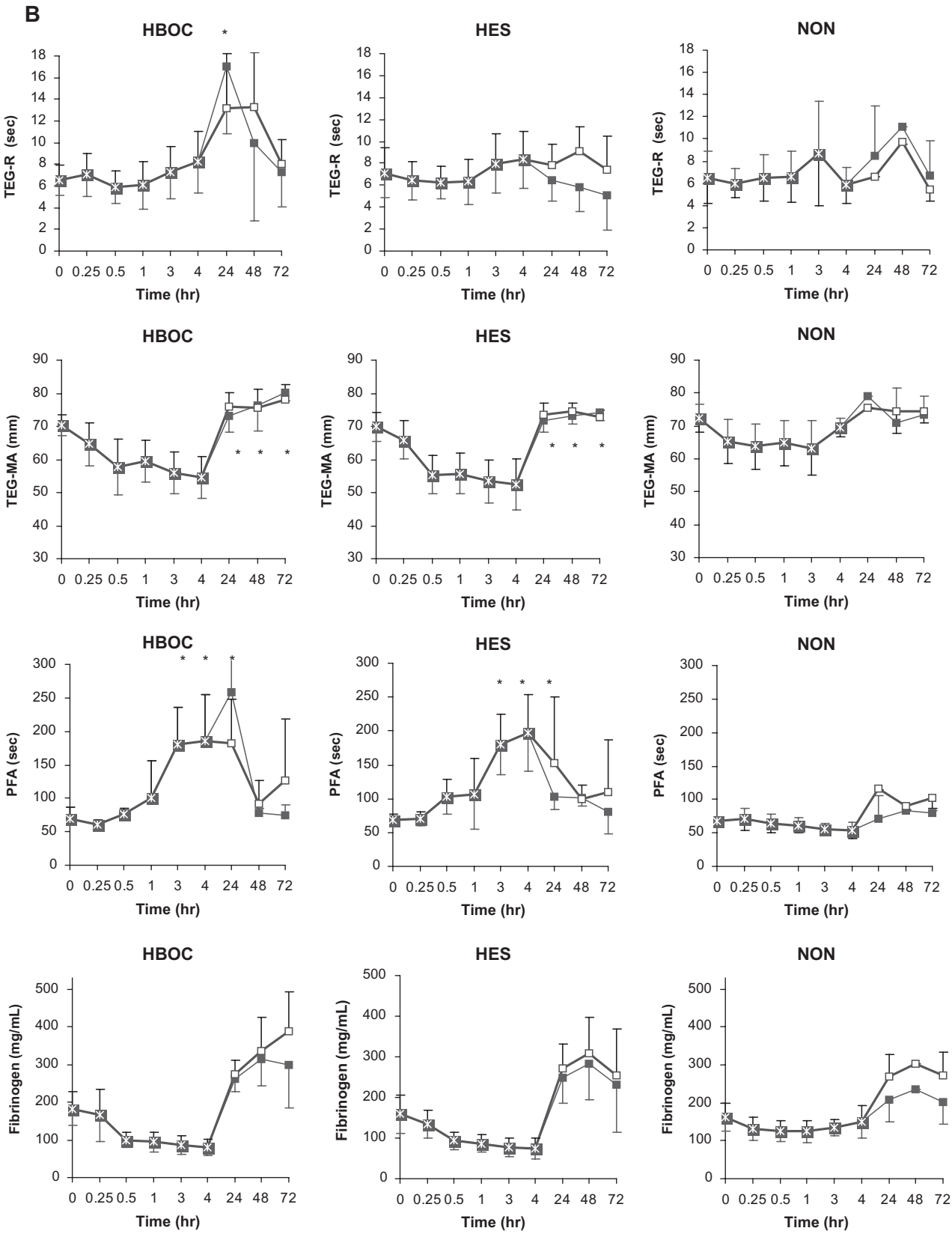

Fig. 4. ***Continued.***

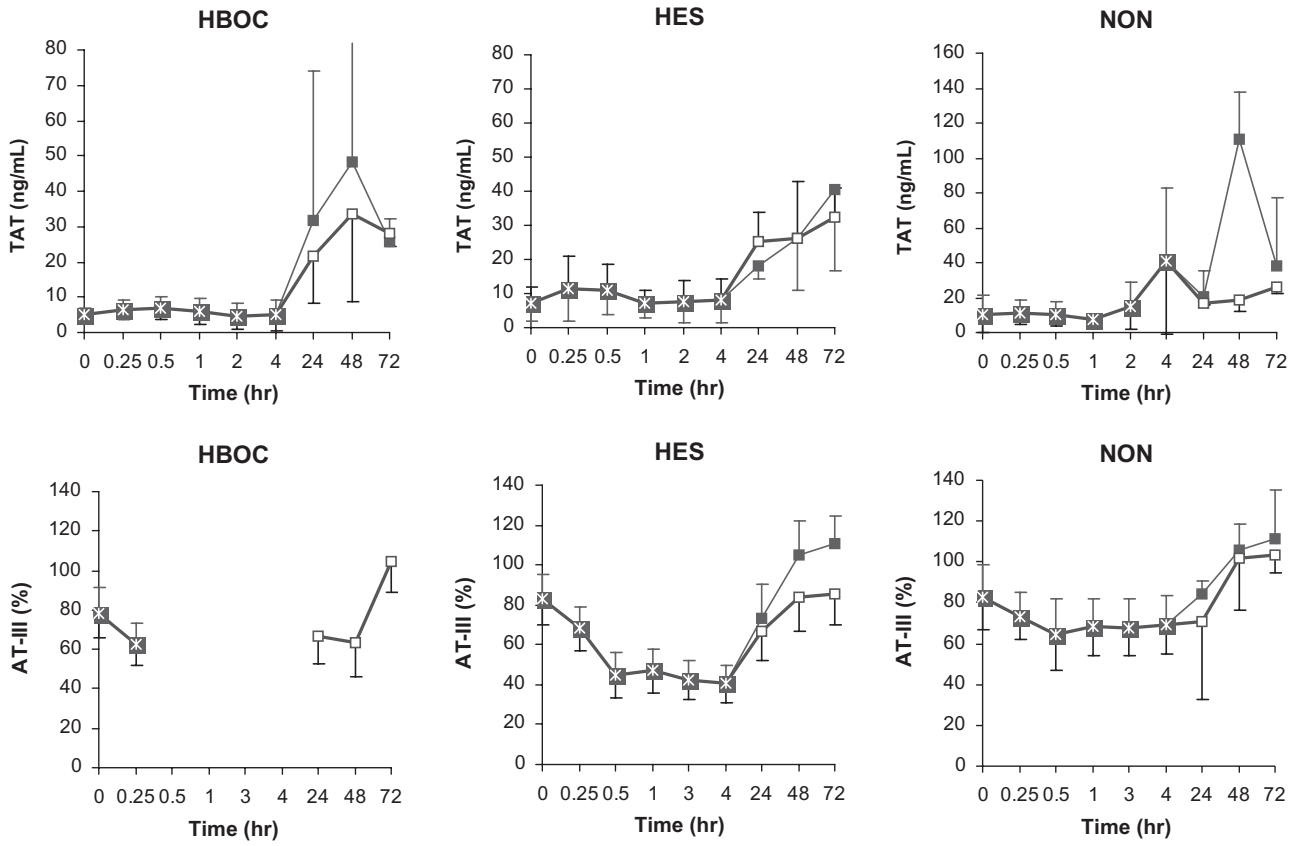

Fig. 4. ***Continued.***

groups during the prehospital phase, but increased in the hospital phase. AT-III decreased after injury and was lower in HEX than NON animals during the prehospital phase; AT-III increased similarly in all groups in the hospital phase. PT was highest with HEX, intermediate with HBOC-201, and lower than in NON animals (unchanged) during the prehospital phase ($p < 0.05$), presumably due to an anticoagulant effect of HEX; PT was similar in all groups in the hospital phase (Fig. 5).

These findings indicated that resuscitation fluid influenced hematology indices in prehospital. At hospital arrival, blood transfusion can restore hematology and coagulation parameters. Animals in the NON group that survived were not hemodiluted and presented an undisturbed pattern for functional parameters. Overall, the prolongation of the delay to hospital arrival did not significantly change the outcomes.

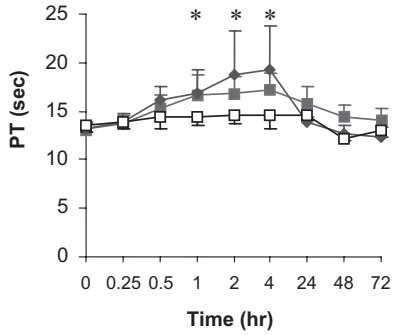

Fig. 5. PT after 55 percent EBV-controlled hemorrhage in the 4- and 24-hour delay cohorts were combined for all treatment groups (■, HBOC-201; ◆, HEX; ◇, NON). *$p < 0.05$ between resuscitated and NON animals.

DISCUSSION

General

Early fluid replacement for patients with hemorrhagic hypotension is paramount for survival especially in the first hour after trauma in both military and civilian settings.[14] Complications including coagulopathy and inflammation, however, may occur consequent to the inadequacies of current standard fluid resuscitation, whether due to intrinsic properties of the fluids or timing or volume effects.[4] This study compared the hemostatic effects of resuscitation with HBOC-201, HEX, and no resuscitation in a swine model of severe controlled hemorrhage (55% blood loss), distinguished by varying the simulated delay to definite care at 4 and 24 hours.

Coagulation

The experiments described coagulation in extreme severity conditions of hemorrhage (55% EBV), as well as delay in hospital arrival (4-24 hr). Overall, differences in hemostatic parameters between the two resuscitation groups were minimal. There was some evidence of coagulopathy in HBOC-201 and HEX animals during the prehospital phase, directly related to fluid infusions. Intrinsic coagulopathic effects of hetastarch-based resuscitative fluids may have been responsible for the increased PT during the prehospital phase with HEX.[15] Conversely, there was more coagulopathy in HBOC-201 animals than HEX animals during the hospital phase due to decreased and delayed need for blood transfusions in HBOC-201 animals. Specifically, TEG-R and PFA were higher with HBOC-201 than with HEX at 24 hours; the mild coagulopathy induced by HBOC-201 resuscitation self-reversed at 48 hours after clearance and/or fluid infusions. Although PT returned to normalcy, AT-III and TAT increased in the hospital phase, indicating a late hemostatic response after hemorrhage involving thrombin production taking place after 4 hours. AT-III and TAT responses were unaffected by differences in resuscitation treatment and evacuation delay. Also evident in the hospital phase was an increase in acute-phase reactant levels (e.g., fibrinogen) exhibiting the same pattern independent of treatment and prehospital delay, consistent with previously published studies.[9,10] In general, the results herein confirm the hematology and coagulation profiles observed in less severe 40 percent EBV-controlled hemorrhage[9] and more severe uncontrolled hemorrhage due to liver injury,[10] with an exception with PFA during the prehospital phase. Specifically, in prior studies, fluid requirements were lower with HBOC-201 than with HEX, resulting in less prehospital coagulopathy with HBOC-201. In contrast, because prehospital fluid requirements were equivalent in the two treatment groups in the studies herein, prehospital PFA profiles were also similar.

Survival

The fact that most of the mortality occurred early in the prehospital phase strongly outlines the importance of fluid resuscitation for survival, and in fact, not surprisingly survival was significantly higher in fluid-resuscitated than non–fluid-resuscitated animals. The observed early mortality also resulted in an absence of a survival difference between the 4- and 24-hour delay cohorts in HBOC-201, HEX, and NON animals.

Compared to standard resuscitation fluids in hemorrhagic shock animal models, HBOC-201 had consistently improved blood pressure and tissue oxygen delivery and decreased blood transfusion requirements when compared to standard resuscitation fluids in hemorrhagic shock animal models.[11,16] The benefit of HBOC-201 in survival, however, has been demonstrated principally in severe models (Grade III liver injury) together with a reduction of blood lactate[6-8] (e.g., increased survival from 12.5 to 87.5 percent for HEX and HBOC-201, respectively)[6] but has not been significantly improved in less severe models (88%-100% for HEX and HBOC-201, respectively).[5] Physiologic results of the studies described herein documented similar patterns. The results herein indicate that the survival of NON-treated animals decreased from 63 to 25 percent, confirming the increased severity of the increased hemorrhage from 40 to 55 percent EBV blood loss. Because survival was 81.7 percent with HEX, it could not be statistically improved with HBOC-201. Surprisingly survival did not decrease with 24-hour delay with HEX reinforcing the role of volume resuscitation in this model rather than fluid properties. Interestingly, delaying definitive care to 24 hours, simulating settings such as rescue in remote or inaccessible areas, only minimally compromised coagulation. The current study addresses the ability of HBOC-201 to function as a prehospital bridging resuscitation fluid and the results suggest that prolongation of delay to definitive care may not affect hemostatic parameters after HBOC-201 resuscitation. We confirmed that even in a situation of increased severity, delayed infusion of HBOC-201 did not contribute to additional coagulopathy and still reduced blood requirement over the 3-day experiments.

Blood transfusion

Our results indicate equivalent survival with a trend to lower prehospital fluid infusion with HBOC-201. Significantly lower blood transfusion requirements after a 24-hour delay to definite care suggest that HBOC-201 may still be an improved resuscitation fluid. The pattern of blood transfusion requirement found between HBOC-201, HEX, and NON treatment groups was consistent with other reports of controlled and uncontrolled hemorrhage swine models.[9,10] At 4 hours, HBOC-201 and HEX animals showed hemodilution and NON animals showed hemoconcentration. The absence of differences in Hb, Hct, and PLTs between in HBOC-201 animals in the two delay cohorts shows that animals ineligible for blood transfusions at 4 hours were not really challenged by the 24-hour delay because few animals required transfusion at 24 hours anyhow. Of the HEX-treated animals that received blood transfusions at 4 hours, few required additional transfusions at a later time. Levy and coworkers[17,18] and King and coworkers[19] have also reported reduction or elimination of RBC transfusions after resuscitation with HBOC in surgical patients.

Data from the medical literature predict that lower blood transfusion requirements and delay in need for blood transfusions would contribute to improved clinical outcome. The literature suggests that blood transfusion is

an independent predictor of adverse outcome in trauma with regard to mortality; MOF, systemic inflammatory response syndrome (SIRS) and infection; and intensive care unit (ICU) admission and length of stay, particularly in bleeding patients. Sauaia and colleagues[20] and Moore and colleagues[21] showed blood transfusion to be an independent risk factor for MOF. Dunne and associates[22] showed blood transfusion to be an independent risk factor for SIRS, ICU admission and length of stay, and mortality. Malone and coworkers[23,24] demonstrated blood transfusion to be an independent and time-dependent (24 hr) risk factor for ICU admission, ICU and hospital length of stay, and mortality. Hill and coworkers[25] and Claridge and coworkers[26] found blood transfusion to be an independent risk factor for postoperative bacterial infection. We recognize that in our study, however, bleeding does not occur and the blood Hb level was used as the transfusion trigger, and thus, the model design criterion dictated the observed transfusion avoidance. Furthermore, the short $t_{1/2}$ of Hb in HBOC-201 (approx. 19 hr) and oxidation to met-Hb may limit oxygen-carrying capacity after 24 hours. Treatment with fresh-frozen plasma should be considered after HBOC-201 or standard fluid resuscitation upon hospital arrival to reverse dilutional coagulopathy. Also, in clinical practice transfusion of red blood cells might still be advisable in HBOC-201–resuscitated patients with uncontrolled hemorrhage to restore hemodiluted cell mass, an important contributor to PLT plug formation and hemostasis.[27,28]

Hemoconcentration in the NON group appeared mainly due to autotransfusion rather than dehydration as Na^+ remained stable in prehospital phase. A splenic autotransfusion of 200 mL is possible;[29] this volume corresponds to an approximately 10 percent increase in Hct in the NON group, a potentially clinically important equivalent to one blood transfusion that may have been but masked by dilution in the other treatment groups. The addition of blood did not translate into a survival benefit in the NON animals, suggesting that even though hemodilution may have detrimental effect in reducing coagulation factors, cell mass, and blood viscosity in the resuscitated groups, it may have reduced building of harmful substances such as proinflammatory cytokines. Hemodilution may exert secondary advantages such as suppressing levels of chemokines,[30] illustrated by the occurrence of lower WBC counts at 4 hours in the resuscitated animals but elevated in the NON animals. Blood products transfusion during the hospital phase in the resuscitated-hemodiluted animals may further reduce harmful chemokines as well as improving their hematology status. It should be recalled that blood transfusions may also be immunoactive and may result in elevation of inflammatory plasma cytokines.[31,32] With blood product transfusions, nonhemolytic febrile reactions may occur due to cytokine buildup.

PLTs

It is interesting to note that although Hct was reduced more significantly in the 55 percent EBV hemorrhage than in the prior 40 percent EBV hemorrhage, PLT counts were reduced equivalently despite the difference in hemorrhage volume in the models in all groups. Thus, it appears that PLT number may be influenced by splenic sequestration and autotransfusion, changes in intravascular volume, and possibly other mechanisms aimed at conserving PLT in severe hemorrhage. Thereafter in the hospital phase, general increase of PLT in all groups regardless of blood transfusions suggests release of sequestrated PLTs or initiation of thrombopoiesis.

Overall, prehospital resuscitation with HBOC-201 provided equivalent survival to HEX, even with a prolonged 24-hour delay to simulated hospital-like care. The fact that four animals survived the 4- or 24-hour delay without fluid resuscitation (NON) before hospital arrival illustrates that certain animals have the capability for natural compensation and were not significantly challenged by the delay itself. In these surviving NON animals, the laboratory indices may initially remain "artificially" closer to baseline levels due to the absence of fluid-induced hemodilution effects; however, physiologic and hemostatic effects might have been observed in a longer survival model. Fitzpatrick and coworkers[33] reported equivalent 5-day survival with no evidence of organ dysfunction in swine resuscitated with HBOC-201 or HEX.[33] Therefore, extending survival time (i.e., >7 days) might provide better evidence for long-term benefits of HBOC-201.

Limits

There are a number of limitations to our study. Our hemorrhage models may not be ideal for evaluation of coagulopathy because coagulopathic effects were minimal in NON groups in all of our studies despite metabolic acidosis, irrespective of hemorrhage volume and whether hemorrhage was controlled or uncontrolled.[9,10] This suggests that there were minimal injury-specific coagulopathic effects in the absence of resuscitation fluid hemodilution, this being possibly a consequence of temperature control.[34,35] This absence of significant coagulopathy contrasts with clinical data in which correlation between injury severity score and coagulopathy has been reported.[36,37] It should be recalled that in clinical practice, trauma patients are often hypothermic postinjury, with active rewarming occurring only with resuscitation by ambulance personnel. True hemostasis is substantiated by absence of hemorrhage after vascular damage and use of a controlled hemorrhage model in this study precluded bleeding; this may be especially important when evaluating a potentially vasoactive resuscitation fluid such as HBOC-201 because spontaneous rebleeding appears to

occur with resuscitation-induced increases in MAP to 100 mmHg.[38] Finally, in our studies animals were under anesthesia, and it has been observed that ketamine has some protective properties towards PLTs.[39,40]

In conclusion, this swine controlled hemorrhagic shock study showed diminished blood transfusion requirements with HBOC-201 in comparison with HEX in models simulating prolonged (4 hr) and severely prolonged (24 hr) prehospital delay. Extension of delay to hospital arrival from 4 to 24 hours did not increase coagulopathic effects with HBOC-201. Importantly, survival was equivalent with HBOC-201 and HEX despite fewer and delayed blood transfusions in HBOC-201 animals. The blood transfusion avoidance, however, resulted in mild dilutional coagulopathy after hospital arrival. Thus, in addition to plasma infusion, in trauma patients resuscitated with HBOC-201 repletion of cellular mass with blood transfusions (normalizing Hct) may be warranted even if oxygen content (Hb) is adequate.

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