Is limited prehospital resuscitation with plasma more beneficial than using a synthetic colloid? An experimental study in rabbits with parenchymal bleeding

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BACKGROUND: Reports of survival benefits of early transfusion of plasma with red blood cells (1:1 ratio) in trauma patients suggest that plasma may be a better fluid to replace Hextend for battlefield resuscitation. We studied possible advantages of prehospital resuscitation with plasma compared with Hextend or albumin in a model of uncontrolled hemorrhage.

METHODS: Male New Zealand white rabbits (3.3 ± 0.1 kg) were anesthetized, instrumented, and subjected to a splenic injury with uncontrolled bleeding. Ten minutes after injury (mean arterial pressure [MAP] < 40 mm Hg), the rabbits received small and equal volumes (15 mL/kg) of rabbit plasma (n = 10), Hextend (n = 10), or 5% human albumin (n = 9) or no fluid. Fluids were administered in two bolus injections (20 minutes apart) and targeted to a MAP of 65 mm Hg. Animals were monitored for 2.5 hours or until death, and their blood losses were measured. Arterial blood samples were collected at different times and analyzed for ABG, CBC, and coagulation tests.

RESULTS: There were no differences in baseline measures among groups. Splenic injury caused similar hemorrhages (9.1 ± 0.4 mL/kg at 10 minutes) and decreased MAP in all subjects. Subsequent resuscitation initiated additional bleeding. At 60 minutes after injury (20 minutes after resuscitation), longer activated partial thromboplastin time and lower fibrinogen concentrations were apparent compared with baseline values with differences among groups. Thrombelastography analysis indicated faster and stronger clot formation with plasma and albumin resuscitation than with Hextend use. Shock indices were increased in all groups, but smaller changes were measured in the albumin group. Total blood loss did not differ among resuscitated rabbits but was higher ($p < 0.05$) than among nonresuscitated animals. Survival rates were 11% (untreated), 40% (Hextend and plasma), and 89% (albumin, $p < 0.05$).

CONCLUSION: Resuscitation with plasma or albumin better preserved coagulation function than did Hextend. However, despite these improvements, plasma resuscitation did not reduce blood loss or improve survival, while albumin administration seemed beneficial. (*J Trauma Acute Care Surg*. 2015;78: 752–759.

KEY WORDS: Prehospital resuscitation; plasma; albumin; Hextend; uncontrolled hemorrhage; rabbit.

Uncontrolled hemorrhage remains the leading cause of potentially preventable death among combat troops. Nearly two third of these deaths (67.3%) occurred as a result of torso injuries with noncompressible bleeding that occurred before ever reaching a medical treatment facility.[1] With no effective hemostatic methods to stop noncompressible truncal hemorrhage, the only option for preventing early mortality at the point of injury is to administer fluid in an attempt to restore blood volume, increase blood pressure, and maintain perfusion of vital organs. The choice and the amount of fluid and how it should be administered have been vigorously debated.[2] The fluid must compensate for plasma volume loss to ensure adequate tissue perfusion and oxygen delivery but should not cause significant hemodilution or disruption of tenuous hemostasis that can exaggerate the hemorrhage. Ideally, it should also replenish blood clotting elements to maintain or enhance the patient's natural hemostatic function, thereby reducing blood loss.[3]

The current recommended guideline for battlefield resuscitation is to administer a limited volume of a synthetic colloid (Hextend) to casualties who are in hypovolemic shock (altered mental status or absent or weak radial pulse). A maximum of 1-L Hextend was to be administered as two 500-mL bolus injections—30 minutes apart—in attempts to restore a normal radial pulse (systolic blood pressure, 90 mm Hg).[4] Hextend is an effective plasma expander that rapidly improves organ perfusion, metabolic deficits, and the hemodynamics of patients in shock. However, Hextend also has detrimental effects on coagulation function as shown by thrombelastography, which have been attributed to its inhibition of clotting factors (FVIII, FXIII and von Willebrand factor) and of platelet function.[5,6] These latter effects of Hextend can increase hemorrhage and elevate the risk of exsanguination of casualties with unstable hemostasis on the battlefield. The survival benefits observed using plasma transfusion along with red blood cells (RBCs) in combat hospitals and civilian trauma centers[7–9] have prompted military officials to consider using plasma as a prehospital resuscitation fluid instead of Hextend.[10]

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The purpose of the present study was to explore potential benefits (i.e., improved hemostasis, reduced hemorrhage and mortality) of using plasma as the only prehospital (initial) resuscitation fluid in an uncontrolled, noncompressible hemorrhage model in rabbits. Plasma was administered according to the same battlefield protocol used for Hextend to resuscitate causalities in shock (described later). The effects of plasma were compared with Hextend and with a single plasma protein fluid (5% albumin), which served as a control. Our hypothesis was that resuscitation with plasma would offer greater advantages—less hemorrhage and better survival—compared with the use of Hextend or albumin.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the US Army Institute of Surgical Research and conducted in compliance with the Animal Welfare Act and implemented Animal Welfare Regulations. All animals received care and were used in strict compliance with *The Guide for the Care and Use of Laboratory Animals*.[11]

Male New Zealand white rabbits, weighing 3.1 kg to 3.4 kg, were acclimated for 1 week before use, and blood samples were obtained and analyzed to ensure their health and normal hemostasis. They were anesthetized and allowed to breathe spontaneously throughout experiments, as described before.[12] Briefly, rabbits were induced by two separate intramuscular injections of fentanyl citrate (0.03–0.05 mg/kg) and midazolam (1-5-2 mg/kg) and prepared for surgery. Surgical anesthesia was produced and maintained by additional intramuscular injections (1 mL) of a mixture (1:1 ratio) of ketamine (10–20 mg/kg) and midazolam (0.5–1 mg/kg) every 15 minutes to 30 minutes as needed. No anesthetic injection was given 10 minutes before the start of the injury/hemorrhage and during the rest of experiment unless it was required (i.e., animal begin to gain conscious). To ensure normal oxygenation, a face mask was placed loosely on each animal's nose, and oxygen gas was delivered at 1 L/min for spontaneous respiration. The marginal ear veins and carotid and femoral arteries were cannulated for intravenous fluid administration, vital sign recording, and blood sample collection, respectively. The rabbits' body temperature was maintained at 37°C to 38°C using a heating blanket.

Next, a midline incision was made, and the gastrointestinal tract was wrapped with laparotomy sponges and retracted to the side, isolating the spleen and its vasculatures. After a 10-minute stabilization, a baseline blood sample was collected, and the pressure was recorded (mean arterial pressure [MAP] ≥ 65 mm Hg required). Next, a deep longitudinal incision was made on the dorsal surface of the spleen, thereby cutting the organ and its vasculature in half. The resulting hemorrhage was allowed to occur freely, and shed blood that pooled in the peritoneal cavity was continuously suctioned and measured by weight. At 10 minutes, rabbits were in hemorrhagic shock and in need of fluid resuscitation. They were randomly divided into four groups, three of which received equal volumes (15 mL/kg) of Hextend (n = 10), 5% human albumin (n = 9), or rabbit fresh frozen plasma (FFP, n = 10). The fourth group received no fluid (n = 9). The surgeon (B.S.K.) was blinded to the type of fluid used in each experiment. Fluids (room temperature) were administered in two bolus injections (each taking 5 minutes to administer and 20 minutes apart) and were targeted to raise the MAP to 65 mm Hg, consistent with the current battlefield resuscitation protocol. Rabbits were monitored for 150 minutes after or until death (i.e., persistent apnea and MAP < 20 mm Hg). Additional blood samples were collected from the rabbits at 1 hour after injury (approximately 20 minutes after resuscitation) and 2.5 hours after injury (survivors only) and assayed for CBC, ABG, standard clotting tests (prothrombin time [PT], activated partial thromboplastin time [aPTT], fibrinogen), and thrombelastography (TEG). The TEG assays were performed in triplicate, and clotting was initiated by adding recombinant tissue factor (Innovin, 1:200 dilution) to fresh blood samples collected without using an anticoagulant.

Hextend and 5% human albumin in buffered isotonic salt solution were purchased from Baxter Healthcare. Freshly prepared rabbit FFP was obtained from Innovative Research (Novi, MI). For plasma collection by Innovative Research, rabbits were anesthetized, a midline incision was made, and the abdominal aorta was isolated and cannulated under sterile conditions. Blood (45 mL) was then collected by gravity into 50 mL sterile conical tubes that each contained 5-mL sodium citrate (3.8%) as anticoagulant (9:1 ratio). The tubes were then centrifuged at low speed and then at high speed to remove the cells and platelets. Clear plasma was then collected, stored in a freezer at −25°C, and shipped to us on dry ice for use. FFP was thawed in a 37°C water bath (no precipitate was seen in thawed plasma) and was filtered (0.4 μm) before it was transfused. A sample was taken and tested for coagulation measures. The results were close to the normal range observed in rabbits (PT, 13.6 seconds; aPTT, 24.6 seconds; and fibrinogen, 186 mg/dL).

Data Analysis

Normality of data distribution was evaluated using the Kolmogorov-Smirnov test. Laboratory data were analyzed by repeated-measures two-way analysis of variance with bigroup tests using the Tukey method. Nonparametric data (blood loss) were analyzed with Kruskal-Wallis test and the bigroup comparison using Dunn's test. Survival outcomes were tested by χ^2 and the log-rank test. Data are expressed as mean ± SEM, and a $p < 0.05$ was considered statistically significant.

RESULTS

Baseline measurements of hemodynamic and hematologic values of the rabbits were within normal ranges, with no significant differences among treatment groups (Table 1). The splenic vascular injuries produced profuse hemorrhage, a significant fall (approximately 50%) in MAP (Fig. 1), and spontaneous hyperventilation (rapid and shallow breathing) in animals within the first 5 minutes after injury. At 10 minutes, bleeding nearly stopped (oozing veins), and the average blood loss during this period (first 10 minutes before resuscitation) was 9.1 ± 0.4 mL/kg, with no difference among groups. The first bolus resuscitation fluid (7.5 mL/kg) was administered at 10 minutes (5 mL/min). The resulting pressure rise (30–40 mm Hg) initiated secondary bleeding that lasted for several minutes. The second

TABLE 1. Arterial Blood Analysis of Rabbits at Different Time Points During Experiment

		Hematocrit, %	Platelet, 1,000/μL	PT, s	aPTT, s	Fibrinogen, mg/dL	pH	Base deficit, mM	Lactate, mM	Glucose, mM	HCO_3, mM	$Paco_2$, mm Hg	K, mM/L	Ionized Ca, mM/L
Baseline*	No fluid	42 ± 0.8	365 ± 28	11.2 ± 0.5	16.9 ± 0.3	318 ± 10	7.34 ± 0.02	−1.9 ± 1.5	3.5 ± 0.8	14.7 ± 0.8	28.6 ± 1.6	56.4 ± 4	2.6 ± 0.04	1.40 ± 0.03
	Hextend	42.6 ± 0.6	483 ± 19	12.3 ± 0.2	17.2 ± 0.2	232 ± 15	7.34 ± 0.01	−2.2 ± 0.7	2.1 ± 0.4	9.9 ± 0.9	29 ± 0.9	58.4 ± 2.5	2.7 ± 0.06	1.44 ± 0.03
	Plasma	42.4 ± 1	444 ± 25	11.6 ± 0.2	17.8 ± 0.4	256 ± 9	7.37 ± 0.02	−3.8 ± 0.6	1.8 ± 0.2	11.4 ± 0.6	30.4 ± 0.8	56.6 ± 2.8	2.5 ± 0.09	1.45 ± 0.02
	Albumin	42.2 ± 0.9	418 ± 41	12.3 ± 0.2	18.5 ± 0.6	237 ± 7	7.36 ± 0.02	−3.9 ± 1	1.8 ± 0.3	11.9 ± 1	30.9 ± 1.1	60.4 ± 2.7	2.7 ± 0.05	1.47 ± 0.03
1 h after	No fluid	34.6 ± 2	276 ± 46	11.9 ± 1.3	17.4 ± 0.6	205 ± 32	7.29 ± 0.02	8.9 ± 1.4	12.9 ± 1.9	25.3 ± 3.4	16.6 ± 1.5	36.6 ± 3.2	4.1 ± 0.3	1.26 ± 0.04
	Hextend	22.1 ± 1.2	268 ± 24	12.6 ± 0.2	22.7 ± 1	118 ± 12	7.32 ± 0.01	5.9 ± 1.3	10.7 ± 1.4	13.7 ± 1.5	19.9 ± 1.4	41 ± 2.6	3.7 ± 0.4	1.28 ± 0.02
	Plasma	25.1 ± 0.9	298 ± 19	12.9 ± 0.2	**20 ± 1****	**168 ± 14****	7.34 ± 0.01	3.7 ± 0.9	9.6 ± 1.1	16.5 ± 1.4	21.7 ± 0.9	42.4 ± 1.4	3.7 ± 0.7	1.19 ± 0.03
	Albumin	**26.1 ± 1.1**†**	**326 ± 17**†**	12.7 ± 0.1	21.3 ± 0.8	133 ± 7	7.35 ± 0.01	**1.8 ± 1.1**†**	7 ± 1.1	18.8 ± 1.7	23.2 ± 1.1	44.7 ± 2	2.9 ± 0.2	1.23 ± 0.02
Final	No fluid	35.8 ± 1.4	210 ± 47	11.7 ± 0.3	17 ± 0.6	225 ± 12	7.00 ± 0.01	16.4 ± 1.4	14.3 ± 2.3	18.3 ± 3.8	14.8 ± 1.7	57.7 ± 12	7.7 ± 1.2	1.31 ± 0.11
	Hextend	23.2 ± 1.9	282 ± 32	12.5 ± 0.2	18.9 ± 0.6	122 ± 11	7.19 ± 0.06	11.2 ± 2.4	9.4 ± 1.2	13 ± 2.9	16.1 ± 1.9	43.8 ± 4.2	6.7 ± 1.3	1.37 ± 0.03
	Plasma	25.1 ± 1	269 ± 34	12.5 ± 0.3	19 ± 0.7	191 ± 17	7.20 ± 0.1	9.5 ± 2.8	8.8 ± 1.1	17.3 ± 2	19.3 ± 0.8	40.3 ± 1.3	5.0 ± 1.1	1.26 ± 0.03
	Albumin	25.6 ± 1.2	315 ± 16	12.9 ± 0.2	23.1 ± 1.7	126 ± 6	7.20 ± 0.07	5.7 ± 2.7	8.8 ± 2.2	19.4 ± 2.2	19.2 ± 1.7	40.8 ± 1.8	4.8 ± 0.9	0.04

*Baseline measurements of resuscitated rabbits were similar among all the three groups. With exception of PT, the baseline measurements were significantly changed ($p < 0.01$) following hemorrhage and resuscitation at 1 hour in all treatment groups.

**†Significantly different ($p < 0.05$) from Hextend-treated rabbits.

Data (mean ± SEM) were analyzed by repeated-measures two-way analysis of variance with b-group tests using the Tukey method. As the purpose of the study was to compare the effect of different resuscitation fluids, the data from nonresuscitated animals (no fluid) were not included in the statistical analyses. Similarly, the final data that could be measured only in the few surviving rabbits were not included in the analysis. Arterial blood samples were collected at baseline (after instrumentation), 1 hour after splenic injury (approximately 20 minutes after fluid resuscitation), and at the conclusion of experiments (final). Because of hypotension, the final blood samples could not be collected from the rabbits that expired during the experiments.

bolus injection was given 20 minutes later when pressure dropped again. MAP at 60 minutes was significantly higher in the albumin group than in other resuscitation groups (Fig. 1).

At 60 minutes (approximately 20 minutes after resuscitation), there were decreases in hematocrit and platelet counts ($p < 0.05$, Table 1); the largest decrease occurred in the Hextend group and the least decrease in the albumin group ($p < 0.05$ vs. Hextend). PT was not changed, but aPTT was prolonged when compared with baseline (Fig. 2*A* and *B*). Numerically, the longest aPTT was measured in the Hextend group ($p < 0.05$ vs. albumin). Fibrinogen concentrations were reduced in all three groups, with numerically the lowest concentrations occurring in the Hextend group and the highest concentration in the plasma group ($p < 0.05$ vs. Hextend, Fig. 2*C*).

TEG analysis showed different coagulation profiles (Fig. 3) based on the type of fluid used for resuscitation. Because there were no differences, the baseline column seen in Figure 3 represents the average baseline of three resuscitated rabbit groups. With the exception of clot strength (MA), all other clotting parameters (*R* time, *K* time, and α angle) when compared with their respective baselines (Table 1), were significantly changed ($p < 0.05$) for each fluid following hemorrhage and resuscitation. The reaction time (*R* time) was reduced after resuscitation with Hextend or albumin when compared with their respective baselines ($p < 0.05$). This value, however, was slightly increased with plasma resuscitation when compared with its baseline and was significantly longer than Hextend or albumin *R* times ($p < 0.05$, Fig. 3*A*). Changes in clot formation time (*K* time) and clotting rate (α angle) indicated a faster clotting process after hemorrhage and resuscitation (Fig. 3*B* and *C*). The fastest clotting was measured in the albumin-resuscitated rabbits as compared with other resuscitated groups ($p < 0.05$). The clot strength (MA) was not changed with albumin or plasma resuscitation compared with baselines, but it was

Figure 1. Averaged MAP of each group of rabbits during the experiment. The data at 120 minutes to 150 minutes represent only the animals that survived. Following a precipitous drop of pressure after injury, each bolus fluid infusion increased MAP and caused rebleeding that lowered MAP to the previous level. At 60 minutes after injury (approximately 20 minute after resuscitation), the MAP of albumin-infused rabbits was significantly higher than that of the other resuscitated animals ($p < 0.05$).

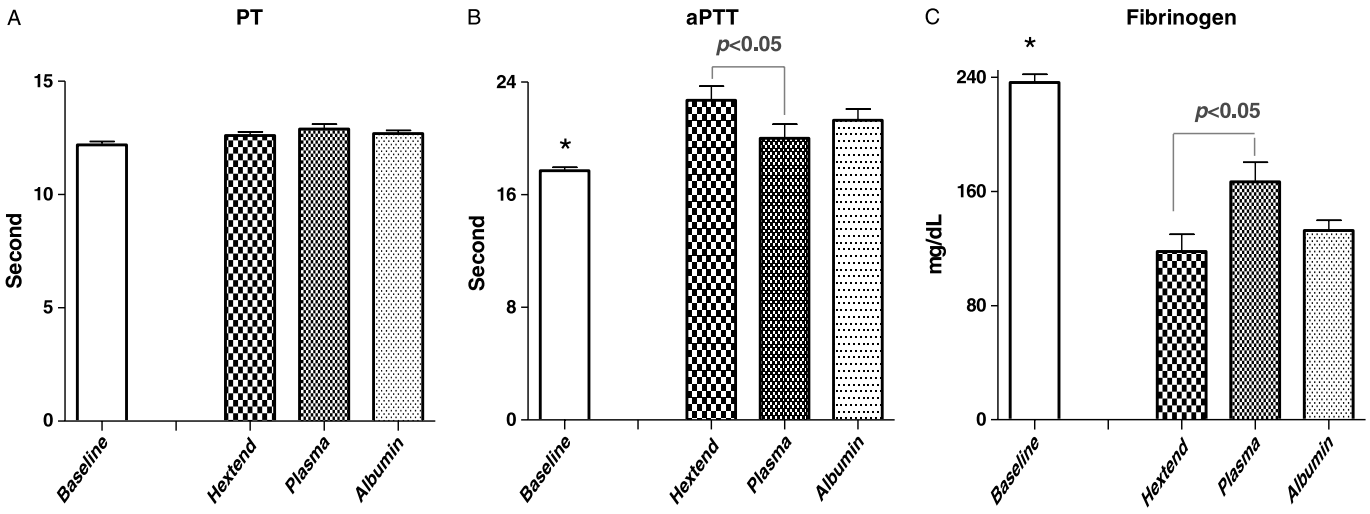

Figure 2. Standard coagulation test results of blood samples collected at baseline and 60 minutes after injury (approximately 20 minutes after resuscitation). There were no differences in baseline measurements among animals. The average for all the three groups is shown in the figure. While PT did not change following hemorrhage and resuscitation, both aPTT and fibrinogen were significantly changed (*$p < 0.05$) as compared with baseline values in all resuscitated rabbits. In addition, the changes in aPTT and fibrinogen in Hextend-treated rabbits were significantly different from those that received plasma.

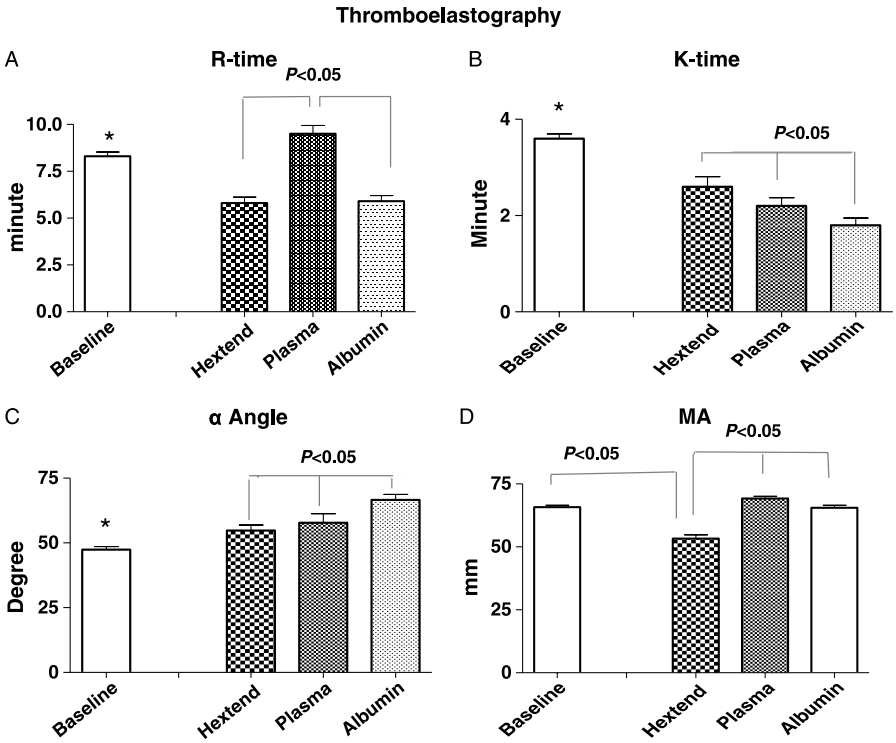

Figure 3. TEG analysis of blood samples collected at baseline and 60 minutes after injury (approximately 20 minutes after resuscitation). The average baseline for all three groups (no differences) is shown in the figure. With the exception of clot strength (MA), all other clotting parameters (*R* time, *K* time, and α angle) were significantly changed following hemorrhage and resuscitation (*$p < 0.05$ vs. baseline values). *R* time was significantly longer with plasma administration than with the other fluids. *K* time and α angle were also changed, indicating faster clotting process in all three groups, with the fastest values measured in the albumin-resuscitated rabbits ($p < 0.05$ vs. other fluids). The clot strength (MA) remained unchanged with albumin or plasma resuscitation, but it was reduced with Hextend administration ($p < 0.05$ vs. baseline and other fluids).

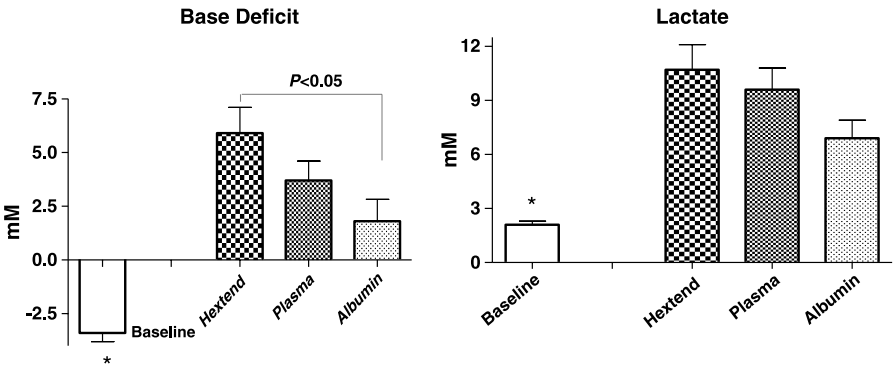

Figure 4. Shock indices measured at baseline and 60 minutes after injury (20 minutes after resuscitation). Both base deficit and lactate levels were significantly increased after hemorrhage and resuscitation (*$p < 0.05$ vs. baseline values). Albumin resuscitation, however, was associated with the least increase of base deficit ($p < 0.05$ vs. Hextend) and lactate levels in the rabbits.

reduced with Hextend administration ($p < 0.05$ vs. baseline and other fluids, Fig. 3*D*). The prolonged aPTT time and significant reduction in clotting rate and clot strength collectively suggested occurrence of a hypocoagulable state following Hextend resuscitation.

At 60 minutes, there were increased levels of base deficit and lactate concentration (shock indices) in the blood of all three groups ($p < 0.05$, Fig. 4). The highest levels were in the Hextend group, and the lowest levels were in albumin-treated rabbits. The difference in base deficit was significant between the albumin and Hextend groups ($p < 0.05$). Total blood loss (before plus after resuscitation), measured at the conclusion of experiments, did not differ among resuscitation groups, and all were greater ($p < 0.05$) than the total blood loss in the untreated (nonresuscitated) animals (Fig. 5*A*). Among those that received fluid resuscitation, postresuscitation blood loss did not differ significantly (Fig. 5*A*). Resuscitated rabbits did not differ in survival times ($p = 0.07$), but they all survived longer than did the nonresuscitated group ($p = 0.001$, Fig. 5*B*). The incidence of survival among albumin-resuscitated rabbits (8 of 9) was greater than incidence of survival in either Hextend or plasma treated rabbits (4 of 10, $p < 0.05$).

DISCUSSION

The present study examined the effect of an early and limited fluid resuscitation on hemostasis and survival of rabbits following a splenic injury and associated uncontrolled hemorrhage. Our goal was to investigate potential benefits of using plasma for simulated prehospital resuscitation of injured subjects with active bleeding compared with a single plasma protein solution (5% albumin) or the synthetic colloid (Hextend) that is currently used on the battlefield. Plasma resuscitation resulted in a survival rate similar to those obtained with Hextend. However, resuscitation with 5% albumin solution led to significantly higher survival rate (89%) compared with the other two groups (40%) and a trend toward improving survival time compared with plasma. This was an unexpected outcome that seemed to be a coincidence since total blood loss was not different

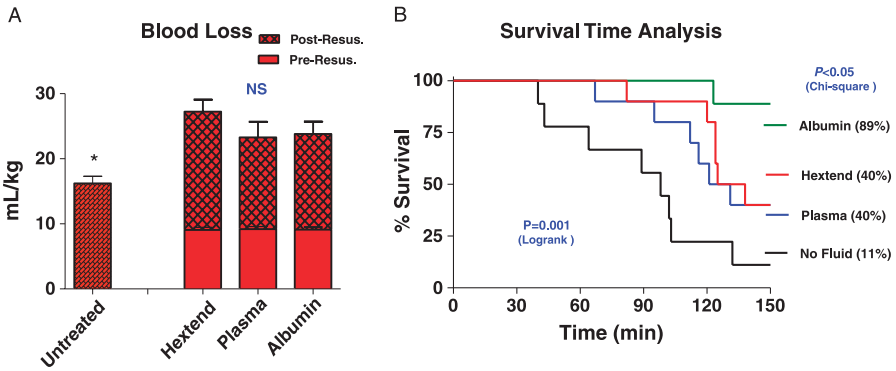

Figure 5. Blood loss (*A*) and Kaplan Meier survival curve (*B*) of nonresuscitated (untreated) and resuscitated rabbits. *A*, Total blood loss (before plus after resuscitation) did not differ among resuscitated groups, but all were greater ($p < 0.05$) than the total blood loss in the untreated animals *B*, Survival times were not significantly different among resuscitated rabbits ($p = 0.07$), but they all lived longer than the untreated animals ($p = 0.001$). The incidence of survival among resuscitated rabbits were significantly different; a larger percentage of albumin-resuscitated rabbits (89% or 8 of 9) survived the duration of the experiment compared with those rabbits that received Hextend or plasma fluid (40% or 4 of 10, $p < 0.05$).

between the plasma and albumin groups. However, we observed similar results when we tested albumin again in a subsequent study in which other colloids and crystalloids were evaluated in the same model.[13] The better tissue perfusion and lower shock indices measured in the albumin group might have been responsible for the survival improvement of rabbits. Other beneficial effects of albumin such as free radical scavenging,[14,15] maintaining microvascular integrity,[16] improving regional blood flow,[17] and neutralizing metabolic acidosis with its strong buffering capacity[17,18] may have also contributed to prolonging rabbit survival here. The albumin advantage was also seen in our previous study in which rabbits were hemodiluted and resuscitated with albumin or Hextend after a splenic injury.[12] In that study, better clotting function, less blood loss, and longer survival times were evident with albumin versus Hextend. Albumin resuscitation was also shown to protect against traumatic/hemorrhagic shock-induced lung apoptosis in rats.[19]

Because plasma contains all coagulation factors and is capable of replenishing the clotting proteins that are lost during hemorrhage, it was expected that infusion of plasma would improve hemostasis and decrease secondary bleeding. However, the results did not fulfill this expectation. Twenty minutes after plasma resuscitation, the only advantage seen was a higher blood fibrinogen concentration; all other hemostatic measurements (PT, aPTT, clotting rate, and clot strength) were not different from those with 5% albumin solution. An apparent disadvantage of using plasma, however, was the reaction time (*R*) that was prolonged in that group. The sodium citrate present in the plasma preparation could not explain this delay as the free Ca^{++} concentration in blood of plasma-treated rabbits was not different from that in other animals. The prolonged reaction time might have been due to natural anticoagulant proteins (e.g., antithrombin, protein C and S) present in the plasma preparation.

The question remains why plasma—which consists primarily of albumin protein (up to 60%)—was less efficacious than pure albumin solution. There are certain known adverse effects associated with plasma resuscitation. For example, in non–massively transfused trauma patients plasma resuscitation was associated with a substantial increase in adult respiratory disorder syndrome, multiple-organ dysfunction, pneumonia, and sepsis.[20] Moreover, although en route plasma resuscitation improved the international normalized ratio of bleeding trauma patients upon arrival to hospitals, no survival benefit was observed in these patients.[21] However, because this study was grossly underpowered and the groups were mismatched, it could not demonstrate a significant survival benefit. Moreover, a small group of trauma patients with lengthy flight times, despite receiving blood products en route, arrived to the hospitals more acidotic than trauma patients who received only crystalloids.[22]

Another finding in this study was that although hemorrhage and subsequent fluid resuscitation resulted in approximately 43% hemodilution (based on hematocrit decrease), PT did not changed significantly, indicating insensitivity of PT test in cases of significant hemodilution. The aPTT, however, was significantly prolonged with the largest change seen in the Hextend group. This result may be due to a larger hemodilution effect, or hydroxylethyl starch (HES) inhibition of factor VIII in blood. Hextend resuscitation also caused a marked decrease in clotting rate (α angle) and clot strength (MA) although the platelet counts and fibrinogen concentrations in this group were not different from those in albumin-treated animals. In another rabbit study, 40% isovolemic hemodilution with Hextend significantly reduced circulating factor VIII:C complex and clotting strength as compared with using a 5% albumin solution. Albumin also resulted in a hypercoagulable state that was attributed to a decrease in blood antithrombin activity.[23]

The previously noted coagulation changes accompanying the use of different resuscitation fluids in the current study did not significantly affect secondary bleeding, although Hextend use led to approximately 25% more blood loss (after resuscitation) than did the other fluids. The least bleeding was measured in nonresuscitated rabbits, but these animals remained hypotensive and died (8 of 9) at earlier times after injury. The findings further support the survival benefit of limited fluid resuscitation at the point of injury even when bleeding has not been controlled.

The use of colloids for small-volume resuscitation has been attractive for the military because of logistical advantages of reduced weight and size relative to crystalloids. For these reasons, Hextend was recommended for prehospital resuscitation of combat casualties.[24] The recent systematic review and meta-analysis of clinical studies comparing HES solutions with crystalloids, albumin, or gelatin, which involved more than 10,000 critically ill patients, indicated that HES administration is associated with a significantly increased risk of mortality and acute kidney injury.[25] These adverse outcomes of HES have prompted the military experts to revise the battlefield fluid resuscitation guideline. The success with damage-control resuscitation (minimum use of crystalloids and early administration of thawed plasma and platelets along with RBC in ratios approaching to 1:1:1)[26] for treating trauma patients has encouraged the military experts to consider the use of blood components for prehospital resuscitation, particularly in circumstances where combat medical personnel must care for casualties in remote locations for several hours or days. However, the technical, regulatory, and logistical constraints prohibit the use of existing blood components in the field. For example, FFP requires freezers for storage at −18°C and the thawing process that not only requires warming equipment but also is time consuming. Plasma as a freeze-dried product (FDP), however, is an attractive alternative that is stable at room temperature and can be reconstituted easily at the point of injury and injected. Hemostatic properties and benefits of FDP or sprayed-dried plasma have been demonstrated in large animal studies with traumatic injuries.[27,28] A small prospective study of FDP in a French intensive care unit with 87 casualties in Afghanistan reported efficacy equivalent to FFP with no adverse effects.[29] As of now, FDP is not yet approved by the Federal Drug Administration, but its use was permitted for military personnel on casualties outside of the continental United States. The Israel Defense forces have selected FDP for fluid resuscitation at the point of injury across all services,[30] and a few US special operation units are carrying a French-made FDP.

Albumin solution has not been considered as a viable solution for battlefield resuscitation because of its possible harm in combat casualties with traumatic brain injury (TBI). The 1998 Cochrane meta-analysis of early clinical studies that compared the effect of albumin with crystalloids in a range of patients concluded that albumin administration was associated with a

significant increase in mortality rate.[31] However, a 2004 multicenter, blinded, randomized, controlled trial reexamined the safety of albumin administration in 6,997 adult intensive care unit patients as compared with saline (Saline versus Albumin Fluid Evaluation, SAFE) found no significant difference between albumin and saline use for either mortality rate or development of organ failure at 28 days.[32] Additional analysis of the SAFE study, however, found that in a subgroup of patients with TBI, albumin administration was associated with a significant increase in mortality rate after 2 years.[33] It should be noted, however, that the SAFE analysis results pertained to the use of a 4% albumin solution in a hypotonic medium (250 mOsm/L) known as Albumex. A 2012 review of the physiology of fluids used for treating TBI patients concluded that osmolality of the fluid rather than its colloid osmotic pressure is the key determinant of cerebral edema formation.[34] The higher mortality rate in the TBI patients resuscitated with Albumex in the SAFE study was attributed to increased intracranial pressure and cerebral edema during the first week after injury.[35] Therefore, the adverse outcomes of albumin resuscitation seen in TBI patients in SAFE study might have been the result of the low osmolarity of Albumex rather than the albumin content of the solution.[34] It should be mentioned that FFP transfusion alone or combined with RBCs were also found to be associated with worse outcomes including long-term disabilities and death in adult and pediatric TBI patients.[36,37]

The albumin solution used in this rabbit study was a 5% human albumin (Albminar) in an isotonic solution (normal saline) with oncotic pressure and pH that approximate those in human plasma. However, administration of human albumin to resuscitate rabbits may be considered either improper or incompatible for such use. That no physiologic adverse event was seen in spontaneously breathing rabbits and that the hemostatic outcomes were even better than using rabbit FFP indicate that at least in the short term, the fluid was both compatible and apparently beneficial in rabbits. It would have been ideal if we could have used rabbit albumin solution instead of human albumin solution in this study. However, a sterile rabbit albumin solution appropriate for intravenous injection is not commercially available. Rabbit albumin is sold by chemical companies as lyophilized powder that is not sterile or may even have some contamination. Neither we nor the company that provided us with rabbit plasma had expertise in fractionating and purifying albumin from rabbit plasma. It may also seem more appropriate to compare the human albumin to human plasma for resuscitation. We have recently tested and determined that fresh human plasma from a single donor or freeze-dried human plasma (the French product prepared from multiple donors suitable for transfusion to any blood type human recipient) are totally incompatible with rabbit RBCs causing severe hemolysis and RBC agglutination. Therefore, such a comparison cannot be made in a rabbit experimental model.

The early start of fluid resuscitation (10 minutes after injury) may be another limitation of this study since such expeditious treatment may not always be possible given the circumstances on the battlefield. In our model, however, the rapid resuscitation seemed necessary because of profuse hemorrhage and the concomitant sharp decline of blood pressure observed in the rabbits, which brought them close to death. We were afraid that further delay in resuscitation might push the animals into irreversible shock after which no fluid would provide adequate resuscitation.

In summary, administration of a small volume of rabbit FFP to resuscitate rabbits following uncontrolled hemorrhage and shock significantly improved hemostatic measurements of the animals compared with those treated with Hextend. However, these changes did not lead to less bleeding or improvement in short-term survival. Similar hemostatic improvements were also achieved with 5% albumin, which additionally reduced base deficit and lactate levels (i.e., neutralize metabolic acidosis) and improved survival. A multicenter study of trauma patients may be warranted to determine the risk and benefits of prehospital resuscitation with plasma versus 5% isotonic albumin solution.

AUTHORSHIP

All authors contributed in the design of the study, developing methodology, and interpretation and editing of the manuscript. B.S.K. performed the surgical procedures, treatments, data collection and analysis, and manuscript preparation, with the technical assistance from K.K.V.-D., I.B.T., and N.M. M.A.D. guided the study and reviewed and edited the manuscript.

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DISCLOSURE

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