

The use of lyophilized plasma in a severe multi-injury pig model

Tim H. Lee, Philbert Y. Van, Nicholas J. Spoerke, Gregory J. Hamilton, S. David Cho, Kate Watson, Jerome Differding, and Martin A. Schreiber

BACKGROUND: Shock and severe tissue injury lead to an endogenous coagulopathy mediated by activation of Protein C and hyperfibrinolysis known as acute traumatic coagulopathy. Together, hemodilution, acidosis, inflammation, and hypothermia result in a global trauma-induced coagulopathy. Coagulopathy in trauma is associated with mortality. Early and effective hemostatic resuscitation is critical in restoring perfusion, correcting coagulopathy, and saving lives in exsanguinating trauma. Lyophilized plasma (LP) provides a logistically superior alternative to fresh frozen plasma (FFP).

STUDY DESIGN AND METHODS: Plasma was lyophilized following whole blood collection from anesthetized swine. A series of studies were performed using anesthetized swine subjected to a validated model of polytrauma and hemorrhagic shock including a Grade V liver injury. Animals were randomized to resuscitation using reconstituted LP fluids. Physiologic data and blood loss were measured. Coagulation status and inflammatory mediators were evaluated.

RESULTS: Full volume reconstituted LP (100%LP) retains on average 86% coagulation factor activity compared to fresh plasma and when used in 1:1 ratios with red blood cells demonstrated superior hemostatic efficacy compared to FFP. Hypertonic LP reconstituted using 50% of the original plasma volume (50%LP) had higher coagulation factor concentrations, was well tolerated in swine, and equally effective compared to 100%LP with respect to physiologic and hemostatic properties. Buffering with ascorbic acid resulted in significant reductions in serum levels of tumor necrosis factor alpha and interleukin-6.

CONCLUSION: By minimizing the volume of reconstituted LP and optimizing its anti-inflammatory properties, an LP resuscitation fluid may be created to provide effective hemostatic resuscitation with superior logistical properties.

INTRODUCTION

Trauma is the leading cause of deaths among civilians between the ages of 1 and 44.¹ Active bleeding from injury to major blood vessels and organs can lead to shock and exsanguination if untreated. Uncontrolled hemorrhage accounts for approximately 40% of trauma deaths.^{2,3} The resultant hypovolemic shock associated with the acute loss of intravascular volume in hemorrhaging patients and concurrent severe tissue injury leads to an endogenous coagulopathy mediated by activation of protein C, hyperfibrinolysis, and consumption of fibrinogen known as acute traumatic coagulopathy (ATC).^{4,6} The propagation of ATC to a global trauma-induced coagulopathy (TIC) is a multifactorial process that includes hemodilution, acidosis, inflammation, and hypothermia.⁷ Coagulopathy in trauma is directly associated with poor outcomes.³ Up to 25% of severely injured patients are significantly coagulopathic on arrival to the hospital. Coagulopathy alone has been associated with a fourfold increase in mortality.^{3,6} Additionally, coagulopathy is associated with significantly greater transfusion requirements, organ failure, infection, and critical care stay.⁸ Patients requiring massive transfusion have 20%-50% mortality.^{9,10} Critical in reversing or minimizing ATC and propagation to TIC is a prompt transfusion of blood products to replace blood volume, oxygen carrying capacity, and the components of coagulation.¹⁰ Traditional transfusion practices limiting the administration of fresh frozen plasma (FFP) to one unit for every four to ten units of red blood cells (RBCs) are insufficient to replace lost coagula-

From the Department of Surgery, Division of Trauma, Critical Care & Acute Care Surgery, Oregon Health & Science University, Portland, Oregon.

Address reprint requests to: Martin A. Schreiber, MD, FACS, Department of Surgery, Division of Trauma, Critical Care & Acute Care Surgery, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Mail Code L-611, Portland, OR 97239-3098; e-mail: schreibm@ohsu.edu.

doi: 10.1111/trf.12039

TRANSFUSION 2013;53:72S-79S.

tion capacity in the setting of exsanguinating trauma.¹¹ Studies initiated by the US military have shown that aggressive and early use of component blood therapy is associated with lower mortality and reduced death from hemorrhage in massively transfused trauma patients. In 2007, a retrospective study of 246 severely injured war fighters treated at a single US Army combat support hospital who required massive transfusion showed a significant decrease in mortality (65% vs. 19%, $p < 0.001$) when patients received transfusion ratios of FFP to RBCs that approached 1:1.2.¹⁰ This landmark study was followed by a large civilian study of 466 trauma patients who received massive transfusion that demonstrated early administration of FFP : RBCs (within 6 hr of injury) with the use of ratios approaching 1:1 was associated with improved overall survival.^{12,13} Though the precise ideal ratio of FFP : RBCs is not yet defined, these studies lend strong support to the early use of high ratios of FFP : RBCs to improve overall survival.¹²⁻¹⁵ Platelets are also a vital component in reversing coagulopathy. In the same multi-institutional civilian study of 466 patients, analysis also demonstrated that plasma and platelet to RBC transfusion ratios were independent predictors of death at 6 hours, 24 hours, and 30 days. Patients who received ratios of pooled platelets : RBCs $> 1:2$ had increased 30-day survival compared with those who received transfusion with the use of lower ratios.¹² Additionally, the use of high ratios of FFP and pooled platelets to RBCs decreased the overall number of RBC units needed in massively transfused patients.¹³ Furthermore, the US Army demonstrated that transfusion with the use of apheresis platelets : RBC ratios $\geq 1:8$ in massively transfused patients resulted in increased survival at 24 hours and 30 days.¹⁶ When converted, the equivalent ratio for pooled platelets : RBCs is 0.75:1. Collectively, these studies have resulted in the current military clinical practice guidelines issued by the Joint Theater Trauma System regarding transfusion practices:

The goal in transfusion of the patient with need for massive transfusion is to deliver a ratio of RBCs to plasma to platelets of 1:1:1.

To achieve this goal, however, a large and rapidly available supply of FFP must be readily accessible. The logistical difficulties associated with the collection, storage, and thawing of FFP for use make meeting these high ratio requirements difficult in many civilian centers and certainly in austere military settings. FFP in addition to its storage limitations must also undergo controlled thawing before use and then must be kept refrigerated after thawing. Additionally, once thawed, FFP has a limited shelf life of ≤ 5 days. Collectively, these cumbersome preparation and storage requirements make FFP unavailable for civilian first responders and far-forward combat personnel.

The concept of lyophilizing human plasma for use in resuscitation was first introduced in World War II. Lyophilization uses a low-pressure, low-temperature, and low-humidity process to convert plasma into a fine lightweight powder. The idea resulted from high demand and concerted efforts to overcome the difficult logistics associated with supplying large volumes of human plasma for use in combat settings. However, due to unacceptable rates of transmission of viral diseases to injured combatants attributed to both the practice of pooling of human plasma and inadequate screening methods, the concept was abandoned.¹⁷ Currently, modern screening methods have significantly reduced the risk of human immunodeficiency virus and hepatitis C transmission.¹⁸ Thus, the concept of lyophilized plasma (LP) has reemerged as an attractive, logistically superior alternative to FFP. In contrast to FFP, once produced, LP is stable and can be stored at room temperature for at least 2 years. Additionally, it is lightweight, easily transported, and can be quickly reconstituted before use.^{18,19} With these superior logistical characteristics, LP has the potential to be a widely accessible alternative to FFP.

Clinical investigations after World War II demonstrated comparable hemostatic effects of LP to fresh plasma as demonstrated by prothrombin time (PT).²⁰ In more recent years, the use of LP has been reported as a treatment option for bleeding hemophilia patients.²¹ Swine models have been utilized to study LP. A feasibility study was performed with the use of a swine model of polytrauma and severe hemorrhagic shock, demonstrating that reconstituted LP was comparable to FFP in reversing coagulopathy as measured by conventional coagulation tests and thrombelastography (TEG).²² Subsequent work by the same group went on to investigate the use of spray-dried plasma due to its reported superior protein viability compared with LP. Spray drying differs from lyophilization in method by the use of heated gas and a volatile solvent to produce a finely powdered product that is highly stable and soluble. The spray-dried plasma was then reconstituted with the use of only one-third of its original plasma volume and demonstrated to have the same efficacy in hemostatic resuscitation as FFP and LP.²³ While these results showed great promise, further studies with the use of spray-dried plasma have not yet been reported.

Having prior experience with the use of LP and an established relationship with lyophilization specialists at HemCon Medical Technologies, Inc., we sought to systematically define the in vitro characteristics and then evaluate the in vivo effects of LP. We initially compared reconstituted LP with FFP by characterizing the in vitro coagulation properties of plasma before lyophilization and then after reconstitution. To create LP, whole blood was collected in sterile fashion by exsanguinating donor swine. Plasma was immediately processed and separated from the whole blood, then frozen without delay, and transported to HemCon Medical Technologies, Inc. for

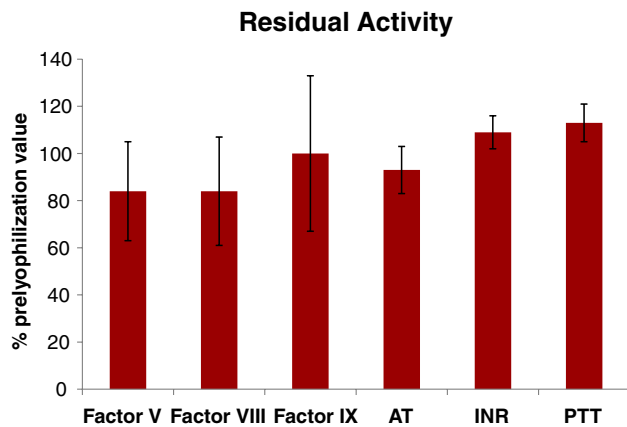


Fig. 1. Clotting factor activity and coagulation assays comparing postreconstitution value with prelyophilization value.

Values presented as mean (standard deviation).

AT = antithrombin III; INR = international normalized ratio; PTT = partial thromboplastin time.

processing into LP. The LP was then returned to our laboratory and reconstituted just before use.

The reconstituted LP was found to be extremely alkalotic and lethal to swine if infused without pH correction. Ascorbic acid (AA) (vitamin C) was chosen for its anti-inflammatory and antioxidant characteristics, as well as its buffering capacity. Following reconstitution of LP to 100% of the original plasma volume (100%LP), the use of sterile water buffered with AA in vitro analysis demonstrated that LP retains on average 86% of the prelyophilization coagulation factor activity.²⁴ Importantly, 100%LP retained 83% of the activity of factor V and factor VIII compared with pre-LP (Fig. 1).

We then proceeded to compare the in vivo hemostatic efficacy of 100%LP with FFP with the use of a validated swine model of multi-injury and severe hemorrhage.²⁵ This complex swine model validated at three separate centers was created to simulate traumatic injury, severe hemorrhage, and subsequent operative intervention with resultant rebleeding. In brief, animals are anesthetized, intubated, and then instrumented for vascular access and hemodynamic monitoring. During the injury phase, an open comminuted femur fracture with severe overlying soft-tissue injury is created with the use of a captive bolt gun. This is followed by controlled hemorrhage with removal of 60% of the animal's total estimated blood volume. Following controlled hemorrhage, the animal is resuscitated with the use of normal saline (NS) given back at three times the volume of shed blood. The animal is simultaneously cooled to 33°C. The combination of severe traumatic injury, hemorrhage, hypothermia, and hemodilution establishes TIC. To simulate operative intervention and rebleeding, a standard Grade V liver injury is created through a laparotomy incision with the use of a modified clamp. The liver is allowed to bleed freely for 30 seconds

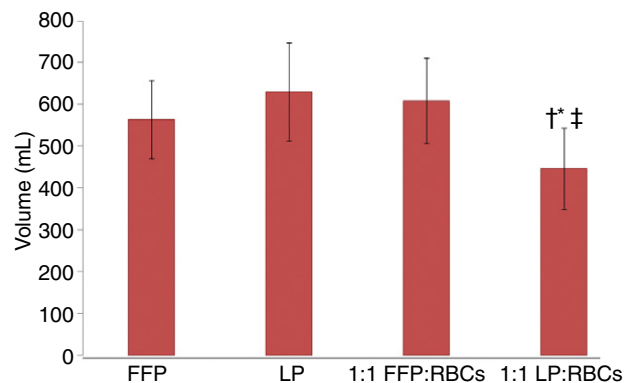


Fig. 2. Total blood loss (eight animals per group). Values presented as mean (standard deviation). *LP : RBCs less than FFP : RBCs group (447 mL vs. 609 mL, $p = 0.006$). †LP : RBCs less than FFP group (447 mL vs. 564 mL, $p = 0.03$). ‡1:1 LP : RBCs less than LP group (447 mL vs. 630 mL, $p = 0.004$). FFP = fresh frozen plasma; LP = lyophilized plasma; RBCs = red blood cells.

following injury after which it is tightly packed with laparotomy sponges and the abdomen is temporarily closed. After the 30 seconds of free bleeding, the animal is randomized and resuscitated with the use of one of the four different resuscitation fluids: FFP, 100%LP, 1:1 FFP : RBCs, or 1:1100%LP : RBCs. The animal is then followed for 4 hours following liver injury.

The results from this study demonstrated no difference in mortality (i.e., all animals survived the 4-hr study period) among the four fluid resuscitation groups. However, there was significantly less blood loss after liver injury in the group receiving 1:1100%LP : RBCs compared with the three other fluids (all $p < 0.03$), suggesting a synergism between LP and RBCs (Fig. 2). Coagulation status of the animals was evaluated by TEG and by measuring the activated clotting time (ACT). The ACT was significantly longer in animals that did not receive RBCs and similarly the TEG R time (time to initial clot formation) was found to be longer in the animals receiving only FFP or 100%LP compared with those receiving fluids with RBCs. These results together support data showing that in addition to soluble clotting factors, RBCs also play a vital role in initial clot formation.²⁶ To assess inflammatory changes, we measured serum levels of interleukin (IL)-6, IL-8, and tumour necrosis factor alpha (TNF- α) throughout the study period with the use of enzyme-linked immunosorbent assay (ELISA). There were no differences in IL-8 or TNF- α among the four fluid groups. However, there were significantly higher levels of IL-6 in the animals resuscitated with FFP compared with those that received 100%LP (all $p < 0.05$), suggesting a possible contribution of AA in attenuating inflammation²⁴ (Fig. 3).

To further investigate the role of AA contained in the 100%LP solution as a mediator in the reduction of trauma-

induced inflammation, we compared 100%LP buffered with AA to 100%LP solutions buffered with either citric acid (CA) or hydrochloric acid (HCl).²⁷ Results from this study demonstrated functional equivalency in terms of overall blood loss with no significant differences occurring among the three study fluid groups. There were also no significant differences in levels of IL-8 and TNF- α among the study fluid groups. However, there was a significantly lower level of IL-6 measured in the groups of animals receiving 100%LP buffered with AA compared with the two other fluid groups (Fig. 4). Hydroxydeoxyguanosine (8-OH-2'-deoxyguanosine [8-OHdG]), a surrogate marker for oxidative damage used in animal models, was also measured throughout the study period with the use of ELISA.²⁸ At 4 hours following liver injury, there was significantly higher 8-OHdG in those animals receiving 100%LP buffered with CA and HCl compared with 100%LP buffered with AA (Fig. 5). Together, these results suggest that AA exerts a significant protective effect against oxidative damage and inflammation in this animal polytrauma model. Possible pathways involved in reducing dysfunctional inflammation may include AA-mediated inhibition of reactive oxygen species and nuclear factor- κ B.^{29,30}

Having established the functional efficacy of 100%LP as a resuscitation fluid with the additional anti-inflammatory properties conferred by addition of AA compared with FFP, we aimed to further optimize LP by minimizing the volume needed for reconstitution. Minimizing the volume would further decrease logistical burdens, thus significantly broadening the availability of this potentially life-saving resuscitation fluid. Thus, we hypothesized that by minimizing the volume needed to reconstitute LP, we can create a low-volume hemostatic resuscitation fluid without loss of hemostatic efficacy. This low-volume LP would make it superior to FFP with respect to logistics, hemodynamic changes, coagulopathy, and blood loss in the established swine polytrauma and hemorrhage model.

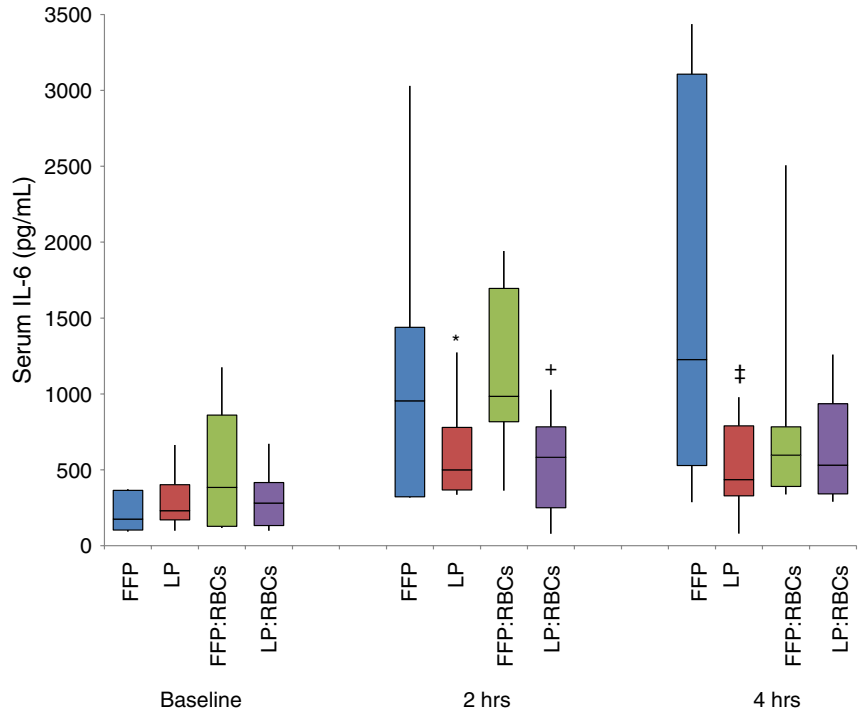


Fig. 3. Serum IL-6 concentrations (eight animals per group). Values presented as medians with interquartile ranges. *LP group less than FFP : RBCs group (p = 0.021). †LP : RBCs group less than FFP : RBCs group (p = 0.009). ‡LP group less than FFP group (p = 0.049). FFP = fresh frozen plasma; IL-6 = interleukin-6; LP = lyophilized plasma; RBCs = red blood cells.

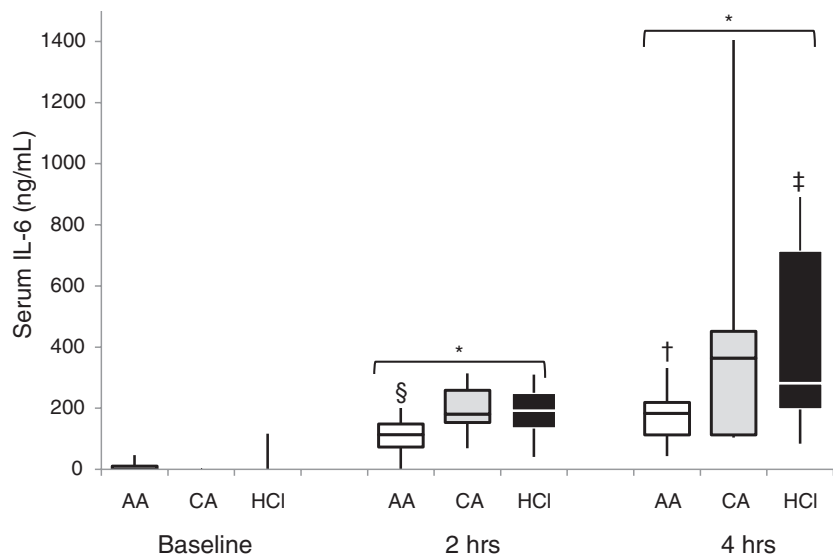


Fig. 4. Serum IL-6 concentrations (10 animals per group). Values presented as medians with interquartile ranges. *All values > baseline. †AA < HCl at 4 hours. ‡HCl > HCl at 2 hours. §AA < CA and HCl at 2 hours; p < 0.05, all comparisons. AA = ascorbic acid; CA = citric acid; HCl = hydrochloric acid; IL-6 = interleukin-6.

To identify the minimal volume sufficient for reconstitution of LP, reconstitution was performed with the use of decreasing volumes of sterile water with AA as buffer. We decreased the volume in stepwise fashion with the use of 10% volume increments. In our hands, LP was successfully reconstituted with the use of a volume of fluid equal to 30% of the original plasma volume. However, animals receiving LP reconstituted to 30 and 40% of the original plasma volume died before or shortly after completion of the LP fluid infusions. All animals survived infusion of LP reconstituted to 50% of the original plasma volume (50%LP). In our experience, the reconstitution of 50%LP took approximately 3 minutes.

The 50%LP solution had significantly higher concentrations of Na, K, Cl, Ca, and albumin compared with 100%LP (all $p < 0.05$). As expected, the 50%LP solution also had a significantly higher osmolarity compared with the 100%LP solution (621 osmol/L vs. 329 osmol/L, $p < 0.05$). The pH of the two study fluids following reconstitution with the use of sterile water with AA as buffer was not different (Table 1).

Regarding coagulation factor activity, there was no significant difference among fresh collected plasma, prelyophilized FFP, and reconstituted 100%LP. However, the 50%LP fluid had increased coagulation factor activity (fibrinogen, II, V, VII, VIII, IX, X, XI, and XII) per unit volume compared with FFP and 100%LP (all $p < 0.03$). Compared with freshly collected plasma that was never frozen, 50%LP did not have significantly higher concentrations of factors II, IX, X, and XI (Table 2).

To compare the efficacy of 50%LP with 100%LP in vivo, the same swine model of polytrauma and hemorrhage was employed. Twenty swine were randomized to receive either 50%LP (n = 10) or 100%LP (n = 10) study fluid. At baseline, animals were similar between the study fluid groups in weight, starting hematocrit (Hct), lactate, and base excess (all p not significant).

Lactate and Hct levels of the animals were measured throughout the injury and hemorrhage model. Lactate levels increased following femur fracture and controlled hemorrhage in both the study fluid groups. Subsequent changes in lactate between groups following liver injury and study fluid resuscitation showed no significant difference. Similarly, Hct decreased following controlled hemorrhage in both the study fluid groups but was not statistically different between the study fluid groups at any time point in the study. There was a predictable decrease

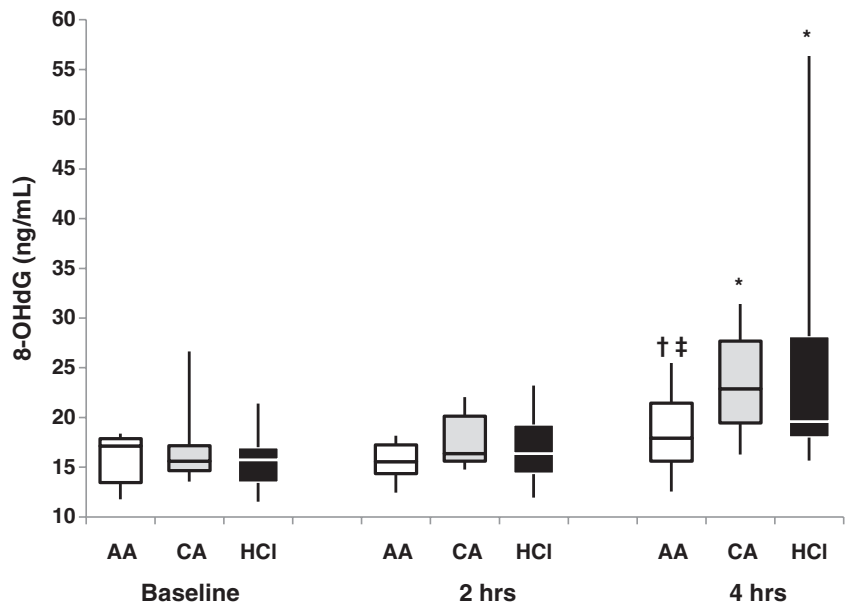


Fig. 5. Oxidative DNA damage represented by median concentrations of 8-OH-2'-deoxyguanosine (8-OHdG) (10 animals per group). Values presented as medians with interquartile ranges. *All values > baseline. †AA < CA at 4 hours. ‡AA > AA at 2 hours; $p < 0.05$, all comparisons. AA = ascorbic acid; CA = citric acid; HCl = hydrochloric acid.

TABLE 1. Baseline characteristics of swine between the study fluid groups

	50%LP (n = 8)	100%LP (n = 8)	p
Na (mmol/L)	297 ± 48	171 ± 22	<0.05
K (mmol/L)	9.2 ± 3.1	4.7 ± 1.3	0.002
Cl (mmol/L)	139 ± 30	80 ± 14	<0.05
Ca (mmol/L)	11.0 ± 2.5	6.5 ± 0.9	<0.05
Alb (mmol/L)	2.0 ± 0.3	1.0 ± 0.2	<0.05
Osm (osmol/L)	621 ± 118	329 ± 44	<0.05
pH	7.11 ± 0.11	7.18 ± 0.08	0.18

Values presented as mean (SD). LP = lyophilized plasma; SD = standard deviation.

in mean arterial pressure (MAP) and increase in heart rate (HR) immediately following femur fracture and controlled hemorrhage. Following the uncontrolled hemorrhage after liver injury, these hemodynamic parameters improved with initiation of both study fluids. There was no significant difference in MAP or HR between the two study fluid groups at any time point during the study (Fig. 6). In terms of blood loss, there was no difference in the 30-second blood loss immediately following liver injury or total blood loss at the end of study from the liver injury between the two study fluid groups. Coagulation status of the animals was evaluated with the use of TEG. Between the two study fluid groups, there was no significant difference noted in TEG parameters at any time point (R time, K, α -angle, or maximal amplitude, all $p > 0.17$).

In summary, this most recent study demonstrated that LP reconstituted to 50% of the original plasma

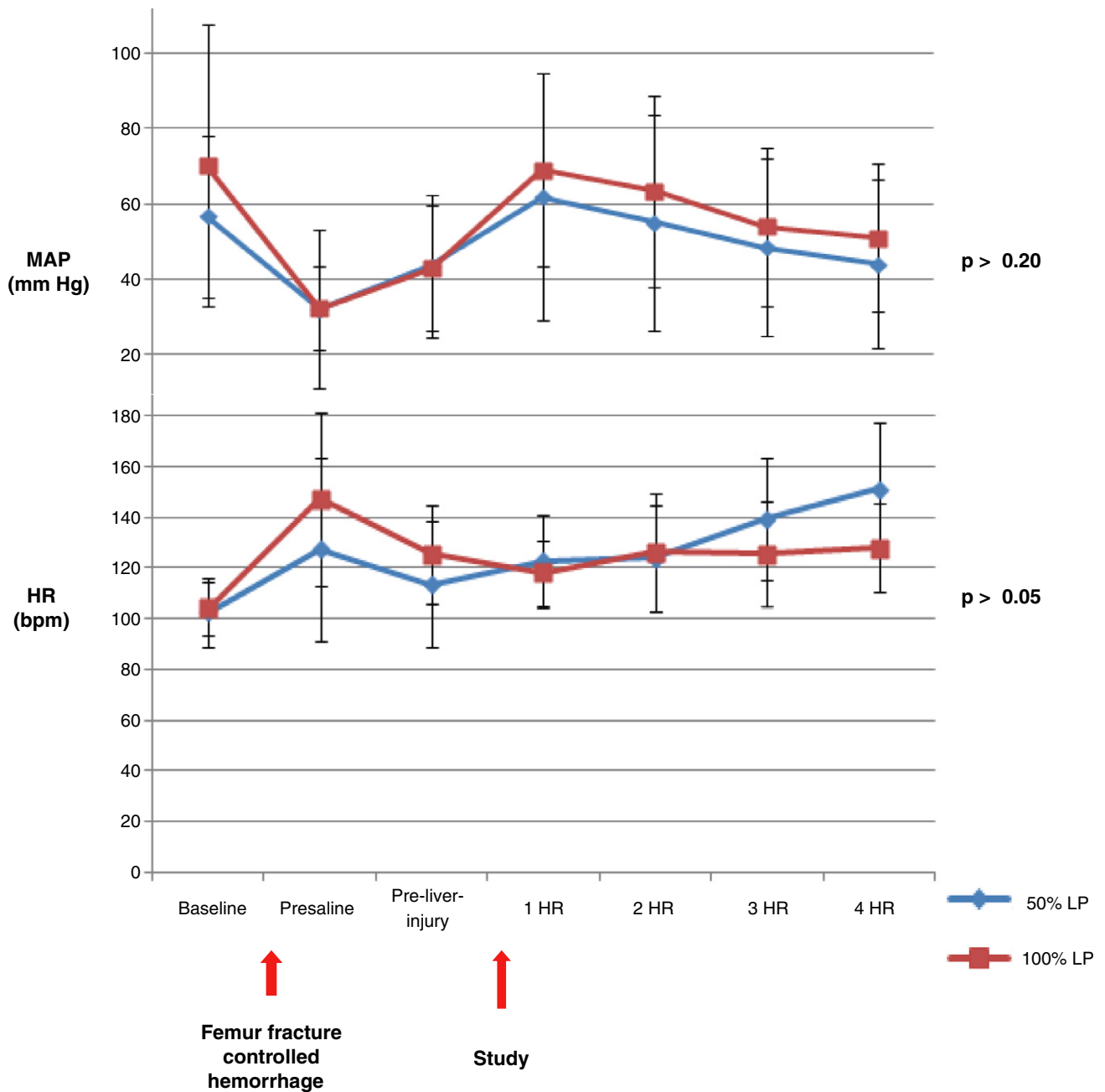


Fig. 6. Hemodynamic changes (10 animals per group). Values presented as mean (standard deviation), all p > 0.05. HR = heart rate; LP = lyophilized plasma; MAP = mean arterial pressure.

volume produces a hemostatic resuscitation fluid with higher coagulation factor activity per unit volume compared with FFP or 100%LP. Though there was no difference in coagulation status between the groups despite the higher concentration of coagulation factor activity in the 50%LP, we propose that the total coagulation factors delivered after complete infusion of both fluids remain equal. The 50%LP formulation thus provides an added advantage in its ability to rapidly restore lost coagulation factor activity in a substantially reduced volume. This is reflected

in the primary outcome, which showed that 50%LP was equally effective in hemostatic resuscitation as 100%LP in terms of measured blood loss.

With the minimal safe volume of reconstituted LP determined and shown to be equally effective in hemostatic resuscitation in an animal multi-injury model, further optimization of the minimal volume 50%LP with the use of alternative reconstitution fluids is being evaluated. In the current prospective, randomized, blinded animal study, we are comparing the standard 50%LP

TABLE 2. In vitro coagulation factor activity. IU/L denotes international units/liter

Factor	Fresh plasma (n = 12)	FFP (n = 16)	100%LP (n = 9)	50%LP (n = 11)
Fibrinogen (mg/dL)	196 ± 46	180 ± 48	170 ± 51	248 ± 41*†
Factor II (IU/L)	46 ± 5	41 ± 7	34 ± 6	51 ± 13*
Factor V (IU/L)	601 ± 91	594 ± 133	550 ± 121	782 ± 223*†
Factor VII (IU/L)	164 ± 22	158 ± 43	136 ± 40	232 ± 77*†
Factor VIII (IU/L)	742 ± 221	607 ± 130	554 ± 149	1024 ± 290*†
Factor IX (IU/L)	284 ± 47	204 ± 35	181 ± 32	330 ± 79*
Factor X (IU/L)	134 ± 19	82 ± 82	73 ± 23	138 ± 50*
Factor XI (IU/L)	117 ± 25	63 ± 16	54 ± 15	136 ± 45*
Factor XII (IU/L)	1304 ± 326	1271 ± 215	1347 ± 213	2223 ± 385*†

Values presented as mean (SD).

* p < 0.05 compared with FFP.

† p < 0.05 compared with fresh plasma.

FFP = fresh frozen plasma; LP = lyophilized plasma; SD = standard deviation.

formulation of sterile water buffered with AA to formulations with the use of either lactated Ringer's (LR), NS, or Hextend buffered with AA. In this active study, animals have been randomized to receive one of the four study fluids (sterile water, LR, NS, and Hextend). Multiple data points are being collected throughout the study: blood loss, hemodynamic parameters, coagulation status, inflammatory response, and oxidative damage will all be evaluated.

Future studies will address maximizing the anti-inflammatory effect of AA by identifying the most effective concentration of AA in LP. Additionally, the use of other antioxidants will be evaluated and tested. Once the 50%LP solution is maximally optimized, animal survival studies will be conducted to evaluate the refined 50%LP solution.

Currently, LP is being used by the Dutch, French, and German armed forces. The US Special Forces has now received approval to use LP for their casualties. A recent prospective study conducted by the French military at the Role 3 Hospital in Kabul, Afghanistan reported on the use of LP in 87 military and civilian casualties (70% Afghani and 30% Coalition).³¹ This study reported an overall mortality of 10% among those patients who received LP, with 67% of these patients in shock when treatment was initiated. This study noted a significant decrease in PT after LP administration in the 36 patients with complete data sets. Though this study was small and many patients were lost to follow-up, there were no reported complications attributable to LP administration. Despite its limitations, this study is the first large-scale report describing the use of LP in injured patients. Currently, in the United States, HemCon Medical Technologies, Inc. has completed Phase I safety trials and is preparing to begin Phase II studies evaluating LP administration in cirrhotic patients and to reverse the effects of coumadin.

Presently, the collective data suggest that LP has great promise as an effective hemostatic resuscitation fluid in prehospital and combat settings. Furthermore, by creating a minimal volume version of reconstituted LP in the form of 50%LP and optimizing its anti-inflammatory proper-

ties, additional reductions in logistical restrictions can be achieved while maintaining maximal efficacy as a hemostatic resuscitation fluid. These superior characteristics have reinvigorated an interest in a decade-old technology. However, now with continuing data-driven preclinical and clinical investigations, LP can potentially return back to the future.

CONFLICT OF INTEREST

None.

REFERENCES

1. Bonne RJ, Fulco C, Liverman CT; Institute of Medicine (US); Committee on Injury Prevention and Control. Reducing the burden of injury: advancing prevention and treatment. Illustrated ed. Washington, DC: National Academies Press; 1999.
2. Sauaia A, Moore FA, Moore EE, Moser KS, Brennan R, Read RA, Pons PT. Epidemiology of trauma deaths: a reassessment. *J Trauma* 1995;38:185-93.
3. Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, Mackway-Jones K, Parr MJ, Rizoli SB, Yukioka T, Hoyt DB, Bouillon B. The coagulopathy of trauma: a review of mechanisms. *J Trauma* 2008;65:748-54.
4. Holcomb JB, Jenkins D, Rhee P, Johannigman J, Mahoney P, Mehta S, Cox ED, Gehrke MJ, Beilman GJ, Schreiber M, Flaherty SF, Grathwohl KW, Spinella PC, Perkins JG, Beekley AC, McMullin NR, Park MS, Gonzalez EA, Wade CE, Dubick MA, Schwab CW, Moore FA, Champion HR, Hoyt DB, Hess JR. Damage control resuscitation: directly addressing the early coagulopathy of trauma. *J Trauma* 2007;62:307-10.
5. Tieu BH, Holcomb JB, Schreiber MA. Coagulopathy: its pathophysiology and treatment in the injured patient. *World J Surg* 2007;31:1055-64.
6. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. *J Trauma* 2003;54:1127-30.
7. Schreiber MA. Coagulopathy in the trauma patient. *Curr Opin Crit Care* 2005;11:590-7.

8. Davenport R, Khan S. Management of major trauma haemorrhage: treatment priorities and controversies. *Br J Haematol* 2011;155:537-48.
9. Malone DL, Hess JR, Fingerhut A. Massive transfusion practices around the globe and a suggestion for a common massive transfusion protocol. *J Trauma* 2006;60 Suppl: S91-6.
10. Borgman MA, Spinella PC, Perkins JG, Grathwohl KW, Repine T, Beekley AC, Sebesta J, Jenkins D, Wade CE, Holcomb JB. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. *J Trauma* 2007; 63:805-13.
11. Ho AM, Karmakar MK, Dion PW. Are we giving enough coagulation factors during major trauma resuscitation? *Am J Surg* 2005;190:479-84.
12. Holcomb JB, Wade CE, Michalek JE, Chisholm GB, Zarzabal LA, Schreiber MA, Gonzalez EA, Pomper GJ, Perkins JG, Spinella PC, Williams KL, Park MS. Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. *Ann Surg* 2008;248:447-58.
13. Zink KA, Sambasivan CN, Holcomb JB, Chisholm G, Schreiber MA. A high ratio of plasma and platelets to packed red blood cells in the first 6 hours of massive transfusion improves outcomes in a large multicenter study. *Am J Surg* 2009;197:565-70.
14. Spinella PC, Perkins JG, Grathwohl KW, Beekley AC, Niles SE, McLaughlin DF, Wade CE, Holcomb JB. Effect of plasma and red blood cell transfusions on survival in patients with combat related traumatic injuries. *J Trauma* 2008;64(2 Suppl):S69-77.
15. Hess JR, Holcomb JB. Transfusion practice in military trauma. *Transfus Med* 2008;18:143-50.
16. Perkins JG, Cap AP, Spinella PC, Blackburne LH, Grathwohl KW, Repine TB, Ketchum L, Waterman P, Lee RE, Beekley AC, Sebesta JA, Shorr AF, Wade CE, Holcomb JB. An evaluation of the impact of apheresis platelets used in the setting of massively transfused trauma patients. *J Trauma* 2009;66(4 Suppl):S77-84; discussion S84-5.
17. Inaba K. Freeze-dried plasma. *J Trauma* 2011;70(5 Suppl): S57-8.
18. Hamilton GJ, Van PY, Differding JA, Kremenevskiy IV, Spoerke NJ, Sambasivan C, Watters JM, Schreiber MA. Lyophilized plasma with ascorbic acid decreases inflammation in hemorrhagic shock. *J Trauma* 2011;71:292-7; discussion 297-8.
19. Daban JL, Clapson P, Ausset S, Deshayes AV, Sailliol A. Freeze dried plasma: a French army specialty. *Crit Care* 2010;14:412.
20. Oktavec WA Jr, Smetana EJ. Lyophilized normal human plasma control in the prothrombin-time clotting test. *Am J Clin Pathol* 1954;24:250.
21. Krutvacho T, Chuansumrit A, Isarangkura P, Pintadit P, Hathirat P, Chiewsilp P. Response of hemophilia A with bleeding to fresh dry plasma. *Southeast Asian J Trop Med Public Health* 1993;24 Suppl 1:169-73.
22. Shuja F, Shults C, Duggan M, Tabbara M, Butt MU, Fischer TH, Schreiber MA, Tieu B, Holcomb JB, Sondeen JL, Demoya M, Velmahos GC, Alam HB. Development and testing of freeze-dried plasma for the treatment of trauma-associated coagulopathy. *J Trauma* 2008;65:975-85.
23. Shuja F, Finkelstein RA, Fukudome E, Duggan M, Kheirbek T, Hamwi K, Fischer TH, Fikry K, deMoya M, Velmahos GC, Alam HB. Development and testing of low-volume hyperoncotic, hyperosmotic spray-dried plasma for the treatment of trauma-associated coagulopathy. *J Trauma* 2011; 70:664-71.
24. Spoerke N, Zink K, Cho SD, Differding J, Muller P, Karahan A, Sondeen J, Holcomb JB, Schreiber M. Lyophilized plasma for resuscitation in a swine model of severe injury. *Arch Surg* 2009;144:829-34.
25. Cho SD, Holcomb JB, Tieu BH, Englehart MS, Morris MS, Karahan ZA, Underwood SA, Muller PJ, Prince MD, Medina L, Sondeen J, Shults C, Duggan M, Tabbara M, Alam HB, Schreiber MA. Reproducibility of an animal model simulating complex combat-related injury in a multiple-institution format. *Shock* 2009;31:87-96.
26. Andrews DA, Low PS. Role of red blood cells in thrombosis. *Curr Opin Hematol* 1999;6:76-82.
27. Van PY, Hamilton GJ, Kremenevskiy IV, Sambasivan C, Spoerke NJ, Differding JA, Watters JM, Schreiber MA. Lyophilized plasma reconstituted with ascorbic acid suppresses inflammation and oxidative DNA damage. *J Trauma* 2011;71:20-4; discussion 24-5.
28. Tomofuji T, Ekuni D, Sanbe T, Irie K, Azuma T, Maruyama T, Tamaki N, Murakami J, Koikeguchi S, Yamamoto T. Effects of vitamin C intake on gingival oxidative stress in rat periodontitis. *Free Radic Biol Med* 2009;46:163-8.
29. Bowie AG, O'Neill LA. Vitamin C inhibits NF-kappa B activation by TNF via the activation of p38 mitogen-activated protein kinase. *J Immunol* 2000;165:7180-8.
30. Hartel C, Strunk T, Bucsky P, Schultz C. Effects of vitamin C on intracytoplasmic cytokine production in human whole blood monocytes and lymphocytes. *Cytokine* 2004; 27:101-6.
31. Martinaud C, Ausset S, Deshayes AV, Cauet A, Demazeau N, Sailliol A. Use of freeze-dried plasma in French intensive care unit in Afghanistan. *J Trauma* 2011;71:1761-4; discussion 1764-5. ■