Different recovery profiles of coagulation factors, thrombin generation, and coagulation function after hemorrhagic shock in pigs

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BACKGROUND: Hemorrhagic shock contributes to coagulopathy after trauma. We investigated daily changes of coagulation components and coagulation function for 5 days in hemorrhaged and resuscitated pigs.

METHODS: Fourteen pigs were randomized into the sham control (C) and the hemorrhage and lactated Ringer's resuscitation (H-LR) groups. On day 1, hemorrhage was induced in the H-LR group by bleeding 35% of the total blood volume, followed by LR resuscitation at three times the bled volume. Pigs in the C group were not hemorrhaged or resuscitated. Hemodynamics and coagulation were measured daily after H-LR on day 1 to day 5.

RESULTS: No changes in hemodynamics and coagulation function occurred in C. Hemorrhage decreased mean arterial pressure and increased heart rate. LR resuscitation corrected these changes within 2 hours. Compared with the baseline values (BL) on day 1, fibrinogen levels were decreased to 76% ± 6% by H-LR on day 1, increased to 217% ± 16% on day 2, and remained increased thereafter; platelet counts were decreased to 63% ± 5% by H-LR on day 1 and remained lower on days 2 and 3 but returned to BL by days 4 and 5 (all $p < 0.05$). Thrombin generation was decreased by H-LR on days 1 and 2 but then increased to above BL on days 4 and 5. Coagulation factor levels were decreased by H-LR on day 1 but returned to BL on day 3 except for factor XIII. Clot strength was decreased by H-LR on day 1 and returned to BL by day 2. Clot rapidity did not change on day 1 but was decreased on days 2 and 3 and returned to BL on days 4 and 5.

CONCLUSION: Hemorrhage and resuscitation reduced coagulation components and compromised coagulation function, which showed different recovery profiles over the 5-day study period. (*J Trauma Acute Care Surg*. 2012;73: 640 647. Copyright © 2012 by Lippincott Williams & Wilkins)

KEY WORDS: Hemorrhagic shock; fibrinogen; platelets; thromboelastography.

Hemorrhage is the leading potentially survivable cause of death on the battlefield and a major cause of death in civilian trauma.[1,2] After blood loss, all components involved in the coagulation process are reduced and further diluted by resuscitation with crystalloid or colloid fluids. To restore coagulation function, different blood products, such as platelet concentrates, cryoprecipitate, or fresh frozen plasma, have been used in patients with bleeding complications.[3–5] However, limited information is available to guide the supplementation of hemostatic components after hemorrhage.

Blood coagulation is an important and complex physiologic process. The essence of blood clotting is the production of fibrin clots catalyzed by thrombin. Thrombin is generated from prothrombin by the activations of platelets, factor VIIa/tissue factor complex, factor Va, and factor Xa. The activation of factor Xa involves the activation of factors VIII, IX, XI, and XII.[6,7] Meanwhile, thrombin generation is subject to inhibition from antithrombin III, thrombomodulin-activated protein C, and tissue factor pathway inhibition. Similarly, clot formation is balanced by the regulation of fibrinolysis via tissue plasminogen activator (t-PA) and t-PA inhibitors. All these components are involved in the complex coagulation process to enable rapid clot formation upon injury and subsequent restoration of hemostasis. After hemorrhagic shock and resuscitation, all coagulation components are decreased because of blood loss and hemodilution. It is not clear whether the decrease and the recovery are similar among all coagulation components. Nor is it clear how coagulation function changes in relation to the recoveries of coagulation components. Furthermore, most trauma patients become hypercoagulable[8,9] rather than hypocoagulable. The mechanisms associated with either form are not well understood. A prospective observational animal study could be helpful to define the changes in coagulation.

This study investigated the daily changing profiles of coagulation factors, platelets, and thrombin generation in relation to coagulation function after hemorrhagic shock and resuscitation using a swine model we developed previously.[10] On inducing hemorrhage and resuscitation with lactated Ringer's (LR) solution on day 1, we measured changes in coagulation components and function daily for 5 days to define the subsequent effects of hemorrhage on the coagulation process.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical

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Research and was conducted in compliance with the Animal Welfare Act and the implementing Animal Welfare Regulations and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals.

A total of 14 crossbred Yorkshire swine (40 ± 2 kg) were randomized into two groups: the sham control group (control, n = 7) and the hemorrhage with LR resuscitation group (hemorrhage, n = 7). After an overnight fast, the animals were preanesthetized with glycopyrrolate (0.1 mg/kg) and Telazol (6 mg/kg), intubated, and anesthetized by 1.0% to 1.5% isoflurane in 100% oxygen by mask for the surgical procedures. Polyvinyl chloride catheters were inserted into the thoracic aorta via the carotid artery for measurement of mean arterial pressure (MAP), heart rate, and temperature. The right femoral artery was cannulated for arterial blood sampling and the left femoral artery for the hemorrhage procedure. The left femoral vein was cannulated for LR resuscitation. The right femoral vein was cannulated for intravenous anesthesia of ketamine during the study. No splenectomy was performed in this study.

On completion of catheter cannulation, anesthesia was switched to a combination of isoflurane (0.5%) and continuous intravenous drip of ketamine (0.15 mL/kg/h of 100 mg/mL) in all pigs for the remainder of the study period. After a 10-minute stabilization period, MAP, heart rate, and temperature were recorded, and blood samples were taken for baseline measurements. Hemorrhagic shock was then induced in the hemorrhage group by bleeding approximately 35% of the total blood volume (24.5 ± 0.1 mL/kg) over about a period of 30 minutes from the left femoral artery to a preweighed canister on a balance. The rate of bleeding was controlled by adjusting the clamp on the left femoral artery catheter to maintain MAP above 40 mm Hg. Afterward, the pigs were resuscitated with LR solution approximately three times the bled volume over approximately 30 minutes. Pigs in the control group were not bled or resuscitated. No shed blood was returned in hemorrhaged pigs. After resuscitation, MAP and heart rate were recorded hourly, and blood samples were taken at 3 hours and 6 hours after resuscitation for measurements of blood gas, blood chemistry, and coagulation. Because no significant changes of coagulation were observed between the 3-hour and 6-hour measurements, the average of the 3-hour and 6-hour measurements was taken as the measurement of day 1 after hemorrhage and resuscitation. After 6-hour blood sampling, the day 1 study was completed, and all catheters inserted during the surgery procedures were taped securely on the pigs' backs. The animals were allowed to awaken and were then transferred to an environmentally controlled room within the vivarium, where they stayed in appropriately sized runs or pens. During the night, the animals were fed with laboratory-grade commercial swine feed by trained animal care staff. Water was provided ad libitum to all animals via an automated water delivery system.

On day 2, the animals were tranquilized with diazepam (0.5 mg/kg intramuscularly) before being transferred to the study room. All catheters were untied and connected to instruments as in day 1 or flushed for blood sampling. After 15 minutes stabilization, MAP, heart rate, and temperature were recorded hourly. Blood samples were withdrawn for coagulation measurements at 3 hours and 6 hours after stabilization. The times of the 3-hour and 6-hour measurements on day 2 coincided with the 3-hour and 6-hour measurements on day 1. After the 6-hour measurements, the pigs were transferred to the vivarium in a cage for the night. The same procedures were performed on days 3, 4, and 5. Because no significant changes in coagulation function were observed between the 3-hour and the 6-hour measurements on days 2, 3, 4, or 5, the average of the 3-hour and 6-hour measurements on these days was presented for the corresponding daily measurements. On completion of the 6-hour blood sampling on day 5, the animals were euthanized with sodium pentobarbital (Fatal Plus, Fort Dodge, IA) given intravenously.

Analytical Methods

Blood gas measurements were determined by the Omni-9 Blood Gas Analyzer (AVL, Montpellier, France). Blood chemistries were measured by the Dimension Clinical Chemistry System (Dade Behring, Newark, DE). Platelet counts were measured from citrated blood using an ABX Pentra 120 Hematology Analyzer (ABX Diagnostics, Irvine, CA). Plasma fibrinogen concentrations, coagulation factors, prothrombin time (PT), and activated partial thromboplastin time (aPTT) were measured using the BCS Coagulation System (Dade Behring, Deerfield, IL). Activated clotting time (ACT) was measured in fresh whole blood samples using Hemochron (HRFTCA 510 Hemochron, International Technique, Edison, NJ).

The coagulation profile was assessed in fresh whole blood samples at the pig's body temperature with tissue factor using thromboelastography (TEG; TEG 5000 Hemostasis Analyzer, Hemoscope Corp, Niles, IL) as described previously.[11] TEG measurements included R time (the time that the initial clot is formed), K time (the time when clot strength reaches a certain level, 20 mm), maximum amplitude (MA) (maximum clot strength), α (the rapidity of fibrin build-up and cross-linking), and LY_{60} (rate of amplitude reduction 60 minutes after MA). TEG measurements were made in triplicate and performed for 1.5 hours to ensure the completion of LY_{60}.

Thrombin generation was determined by quantifying thrombin-antithrombin III (TAT) complex from minimally altered fresh whole blood samples, following the procedure described by Rand et al.[12] Briefly, fresh whole blood samples were added into tubes set on a shaker plate. After 20 minutes, a "quench" solution of 50 mmol/L ethylenediaminetetraacetic acid and 10 mmol/L benzamidine in saline buffered with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid was added to the blood samples to stop clot formation. The quenched samples were centrifuged, and supernatants were collected for TAT concentration measurement using commercially available enzyme-linked immunosorbent assay kits (Enzygnost TAT, Dade Behring, Deerfield, IL). The TAT concentrations from the supernatant samples reflect thrombin content generated from fresh whole blood samples before the addition of the quench solution. This method has been used successfully in our previous studies.[10,13–15]

Statistical Analysis

Data were expressed as means ± SEM and analyzed using SAS statistical software (Cary, NC). A two-way analysis of variance with repeated measures was used to compare the changes

TABLE 1. Changes of Hematocrit, Red Blood Cell Count, and Hemoglobin Levels After Hemorrhage and Lactated Ringer's Resuscitation

	Day 1					
	Baseline	**After H/LR**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Hct (%)						
Control	30 ± 2	30 ± 1	29 ± 2	28 ± 2	26 ± 2	27 ± 2
Hemorrhage	28 ± 1	20 ± 1*†	19 ± 1*†	18 ± 2*†	19 ± 1*†	18 ± 2*†
RBC ($10^6/\mu L$)						
Control	6.1 ± 0.3	5.8 ± 0.1	5.7 ± 0.2	5.6 ± 0.4	5.4 ± 0.4	5.5 ± 0.4
Hemorrhage	5.9 ± 0.2	3.8 ± 0.4*†	3.4 ± 0.2*†	3.3 ± 0.4*†	3.4 ± 0.2*†	3.4 ± 0.3*†
Hgb (g/dL)						
Control	9.8 ± 0.6	9.6 ± 0.3	9.5 ± 0.6	8.6 ± 0.6	8.4 ± 0.8	8.7 ± 0.7
Hemorrhage	9.2 ± 0.2	6.5 ± 0.5*†	6.0 ± 0.3*†	5.7 ± 0.3*†	5.7 ± 0.3*†	5.8 ± 0.4*†

Hct, hematocrit; RBC, red blood cell; Hgb, hemoglobin; H/LR, hemorrhage and lactated Ringer's resuscitation.
* $p < 0.05$ compared with day 1 baseline values within the group, N 7/group.
† $p < 0.05$ compared with corresponding control values.

over time between the two groups. The variance/covariance structure of the model was chosen based on the lowest Bayesian information criterion. The statistically significant level was set at $p < 0.05$.

RESULTS

Hemodynamics

All the animals from both groups survived to the end of the 5-day study. All baseline measurements on day 1 were similar between the control group and the hemorrhage group. No significant changes were observed in hemodynamics in the control group during the 5-day study period based on repeated measures model. In the hemorrhage group, MAP was decreased by hemorrhage on day 1 from baseline value (BL) of 95 ± 4 mm Hg to 53 ± 4 mm Hg ($p < 0.05$) but returned to BL after LR resuscitation within 2 hours. Heart rate was increased by hemorrhage from a BL of 91 ± 8 bpm to 109 ± 13 bpm on day 1 ($p < 0.05$) but returned to BL within 1 hour after LR resuscitation. No significant changes in MAP or heart rate occurred during the remaining study period in the hemorrhage group. No significant changes in body temperature were observed in either animal group during the study.

In the hemorrhage group, blood lactate level was increased by hemorrhage from BL of 1.8 ± 0.1 mmol/L to 2.4 ± 0.2 mmol/L ($p < 0.05$) but returned to 1.7 ± 0.2 mmol/L after LR resuscitation. No further changes in blood lactate levels occurred during the remaining study period. There was no significant change in arterial blood pH in either group during the 5-day study period based on repeated measures model. Hematocrit was decreased by hemorrhage and resuscitation from BL of 28% ± 1% to 20% ± 1% on day 1 ($p < 0.05$) and remained reduced on days 2, 3, 4, and 5 (Table 1). Similarly, red blood cell count and hemoglobin level were decreased by hemorrhage and LR resuscitation on day 1, and remained lower on days 2, 3, 4, and 5 (Table 1). No significant changes in lactate level, hematocrit, red blood cell, or hemoglobin were observed in the control group based on repeated measures model.

Fibrinogen, Platelet, and Coagulation Factors

In the control group, plasma fibrinogen concentration did not change on day 1, increased from the day 1 BL of 180 ± 7 mg/dL to its peak of 340 ± 30 mg/dL on day 2 ($p < 0.05$), decreased to 289 ± 19 mg/dL on day 3 ($p < 0.05$), and returned to the day 1 BL on days 4 and 5. In the hemorrhage group, the fibrinogen concentration was reduced by hemorrhage and LR resuscitation from BL of 170 ± 10 mg/dL to 127 ± 17 mg/dL on day 1 ($p < 0.05$), was increased to 363 ± 40 mg/dL on day 2 ($p < 0.05$), and remained increased on days 3, 4, and 5. No significant changes in platelet count occurred in the control group during the study based on repeated measures model. In the hemorrhage group, platelet counts decreased from BL of 354 ± 44 $10^3/mm^3$ to 223 ± 16 $10^3/mm^3$ after hemorrhage and LR resuscitation on day 1 ($p < 0.05$), remained at the lower levels on days 2 and 3, but returned to BL on days 4 and 5.

There were no significant changes in coagulation factors in the control group during the 5-day study based on repeated measures model. Changes in coagulation factor levels in the hemorrhage group are summarized in Table 2. Hemorrhage and LR resuscitation reduced all of the measured coagulation factors about 30% to 70% on day 1. Factors II, V, VII, VIII, IX, X, XI, and XII remained significantly lower on day 2 but returned to day 1 BL on days 3, 4, and 5 (Table 2). However, factor XIII remained below BL on days 2, 3, 4, and 5 (Table 2).

Thrombin Generation

There were no significant changes in thrombin generation in the control group during the 5-day study period based on repeated measures model. In the hemorrhage group, thrombin generation (plateau values at a 15-minute quench time) was significantly decreased by hemorrhage and resuscitation on day 1, remained lower on day 2, returned to day 1 BL on day 3, and increased above day 1 BL on days 4 and 5 (Fig. 1).

Coagulation Functional Assessments

No significant changes in PT or aPTT were observed in either group during the 5-day study period (Table 3) based on repeated measures model. There were also no significant changes in ACT in the control group during the 5-day study

TABLE 2. Changes of Coagulation Factors After Hemorrhage and Lactated Ringer's Resuscitation in Pigs

	Day 1*	Day 2	Day 3	Day 4	Day 5
Factor II (%)					
Control	92 ± 13	105 ± 5	97 ± 4	112 ± 14	106 ± 5
Hemorrhage	67 ± 3†‡	73 ± 1†‡	101 ± 3	105 ± 4	111 ± 6
Factor V (%)					
Control	94 ± 5	99 ± 6	112 ± 11	115 ± 11	111 ± 13
Hemorrhage	69 ± 5†‡	80 ± 5†‡	114 ± 10	108 ± 5	124 ± 15
Factor VII (%)					
Control	95 ± 6	87 ± 17	103 ± 13	118 ± 12	112 ± 16
Hemorrhage	57 ± 3†‡	65 ± 5†‡	104 ± 10	116 ± 11	118 ± 14
Factor VIII (%)					
Control	100 ± 6	112 ± 11	96 ± 7	98 ± 6	110 ± 8
Hemorrhage	66 ± 11†‡	75 ± 13†‡	85 ± 14	95 ± 11	101 ± 13
Factor IX (%)					
Control	94 ± 6	105 ± 10	109 ± 10	119 ± 16	118 ± 16
Hemorrhage	54 ± 6†‡	62 ± 3†‡	113 ± 11	114 ± 12	118 ± 11
Factor X (%)					
Control	92 ± 12	103 ± 11	109 ± 9	101 ± 4	114 ± 13
Hemorrhage	62 ± 6†‡	84 ± 7†‡	116 ± 8	113 ± 11	117 ± 13
Factor XI (%)					
Control	95 ± 7	107 ± 7	102 ± 12	109 ± 9	110 ± 8
Hemorrhage	37 ± 5†‡	43 ± 7†‡	83 ± 13	88 ± 15	83 ± 12
Factor XII (%)					
Control	94 ± 7	102 ± 6	103 ± 16	110 ± 9	111 ± 8
Hemorrhage	64 ± 7†‡	72 ± 8†‡	85 ± 16	86 ± 10	84 ± 16
Factor XIII (%)					
Control	98 ± 4	92 ± 6	96 ± 6	97 ± 5	94 ± 5
Hemorrhage	70 ± 4†‡	68 ± 7†‡	69 ± 4†‡	77 ± 4†‡	76 ± 5†‡

* Data were measured after hemorrhage and LR resuscitation on day 1.
† $P < 0.05$ compared with day 1 baseline values within the group.
‡ $P < 0.05$ compared with corresponding control values.
Data are expressed as % of corresponding day 1 baseline values in each group (n 7).

period. Compared with the day 1 BL (97 ± 3 seconds), ACT in the hemorrhage group did not change after hemorrhage and resuscitation on day 1, but was prolonged to 116 ± 4 seconds and 109 ± 2 seconds on days 2 and 3 (both $p < 0.05$), respectively, and returned to BL on days 4 and 5.

Compared with day 1 BL, no significant changes were observed in any TEG variables in the control group during the 5-day study period based on repeated measures model. In the hemorrhage group, the initial clotting time (R time) did not change from the BL (3.1 ± 0.2 minutes) after hemorrhage and resuscitation on day 1, but was prolonged to 4.6 ± 0.2 minutes on day 2, remained prolonged on days 3 and 4, and returned to near the BL on day 5 (all $p < 0.05$, Fig. 2). Time to maximum clot (K time) did not change from BL (1.1 ± 0.1 minutes) after hemorrhage and resuscitation on day 1, but was prolonged to 1.8 ± 0.2 minutes and 1.6 ± 0.1 minutes on days 2 and 3, respectively (both $p < 0.05$), and returned to BL on days 4 and 5 (Fig. 2). Clot strength (MA) decreased from BL of 74 ± 2 mm to 66 ± 1 mm after hemorrhage and resuscitation on day 1 ($p < 0.05$) and returned to day 1 BL on day 2 and afterward (Fig. 3). Clotting rapidity (α) did not change from BL of 76 ± 1° by hemorrhage and resuscitation on day 1, decreased to 68 ± 1° and 70 ± 1° on days 2 and 3, respectively (both $p < 0.05$), but returned to the BL on days 4 and 5 (Fig. 3). No significant changes in LY_{60} (fibrinolysis) were observed in either group during the study period based on repeated measures model.

DISCUSSION

Using a swine model, we investigated the daily changes in the coagulation process for 5 days after hemorrhagic shock. A moderate hemorrhage and LR resuscitation caused different changing profiles on hemodynamics and coagulation. Although hemodynamics was recovered within 2 hours, the effects of hemorrhage and LR resuscitation on coagulation lasted as long as 5 days. In addition, hemorrhage and LR resuscitation on day 1 reduced fibrinogen concentration, platelet counts, and coagulation factor levels. Different recovery profiles among coagulation components were observed on days 2, 3, 4, and 5, together with different recoveries of coagulation functional aspects.

Fibrinogen and platelets are two important components in the coagulation process, and different recovery profiles were observed in this study. Hemorrhage and LR resuscitation caused a decrease of about 25% in fibrinogen concentration. By the next day (day 2), fibrinogen concentrations were double the prehemorrhage levels and remained increased on days 3, 4, and 5.

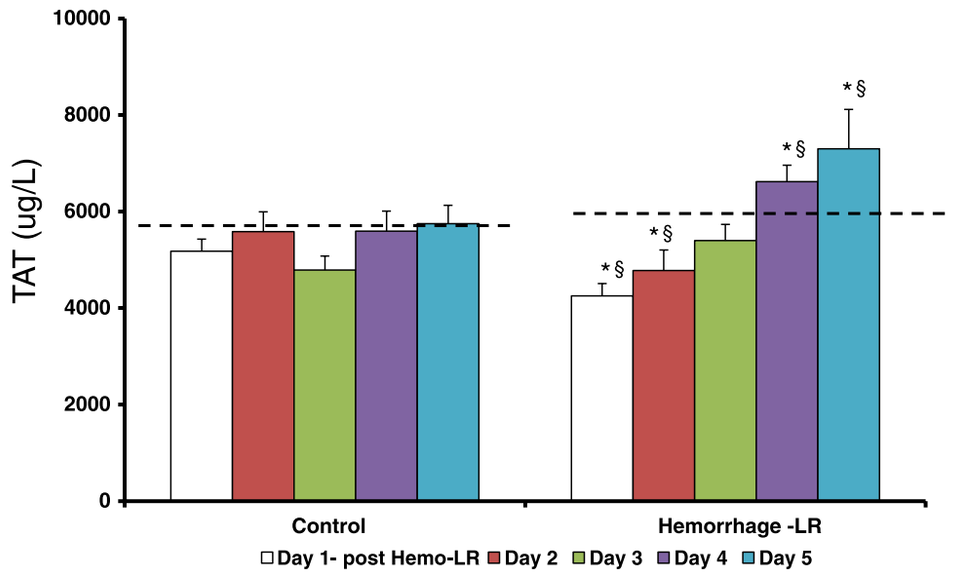

Figure 1. Changes in thrombin generation after hemorrhage with lactated Ringer's (LR) resuscitation in pigs. Data are represented as mean ± SEM for seven animals in the hemorrhage group. *$p < 0.05$ compared with day 1 BL *(dashed lines)* within the group. §$p < 0.05$ compared with corresponding control values.

TABLE 3. Changes of PT and aPTT After Hemorrhage and Lactated Ringer's Resuscitation

	Day 1					
	Baseline	**After H/LR**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
PT (s)						
Control	10.7 ± 0.4	10.7 ± 0.3	10.3 ± 0.2	9.9 ± 0.2	9.8 ± 0.3	9.9 ± 0.1
Hemorrhage	10.8 ± 0.6	10.7 ± 0.4	10.3 ± 0.3	9.8 ± 0.2	9.9 ± 0.2	9.9 ± 0.2
aPTT (s)						
Control	16.6 ± 0.5	16.4 ± 0.2	16.4 ± 0.3	16.0 ± 0.2	16.1 ± 0.2	16.3 ± 0.5
Hemorrhage	16.2 ± 0.4	16.6 ± 0.4	16.3 ± 0.4	16.1 ± 0.2	15.8 ± 0.2	15.8 ± 0.3

PT, prothrombin time; aPTT, activated partial thromboplastin time; H/LR, hemorrhage and lactated Ringer's resuscitation.
N 7/group.

On the other hand, hemorrhage and LR resuscitation decreased platelet counts about 35% on day 1, and platelet counts remained lower on days 2 and 3. It was on days 4 and 5 that platelet counts returned to prehemorrhage levels. Thus, after hemorrhage and resuscitation, the recovery of fibrinogen is faster than that of platelets. The fast recovery of fibrinogen may reflect its priority role in restoring hemostasis after hemorrhagic shock, as well as its roles as an acute phase protein.

Hemorrhage and LR resuscitation caused about 30% to 70% decreases of all measured coagulation factors on day 1 in this study. Coagulation factor II, V, VII, VIII, IX, X, XI, and XII returned to prehemorrhage values on day 3. A similar recovery pattern was observed in thrombin generation. Thrombin generation was decreased on day 1 by hemorrhage and resuscitation and returned to day 1 BL on day 3. Thrombin generation requires the activation of factor VIIa/tissue factor complex, platelets, factor Va, and Xa, and the activation of factor Xa involves the activation of factors VIII, IX, XI, and XII.[6] Thus, the similar recovery patterns between thrombin generation and coagulation factors may reflect that thrombin generation is closely related to the availabilities of these coagulation factors.

Similar sequential changes in coagulation factors were reported previously in a canine model and in patients with hemorrhagic shock and blood transfusion.[16,17] In dogs with hemorrhagic shock followed by resuscitation with LR solution and volume exchanges, Lucas et al.[18] reported that the fibrinogen level was decreased by hemorrhage and resuscitation and increased on the next day and that levels of factor II, V, VIII, and X were decreased on the hemorrhage and resuscitation day and returned to BL 48 hours later. Similarly, in patients with hemorrhagic shock and blood transfusion, Harrigan et al.[16] reported that the fibrinogen level decreased during operation but was increased and plateaued on day 2 after operation and that factor V and VIII levels were decreased during operation and returned to normal values on day 2. Correspondingly, PT and aPTT in those patients were prolonged during operation and returned to normal values on day 2,[16] reflecting the changes in coagulation factors. The lack of changes in PT or aPTT in this

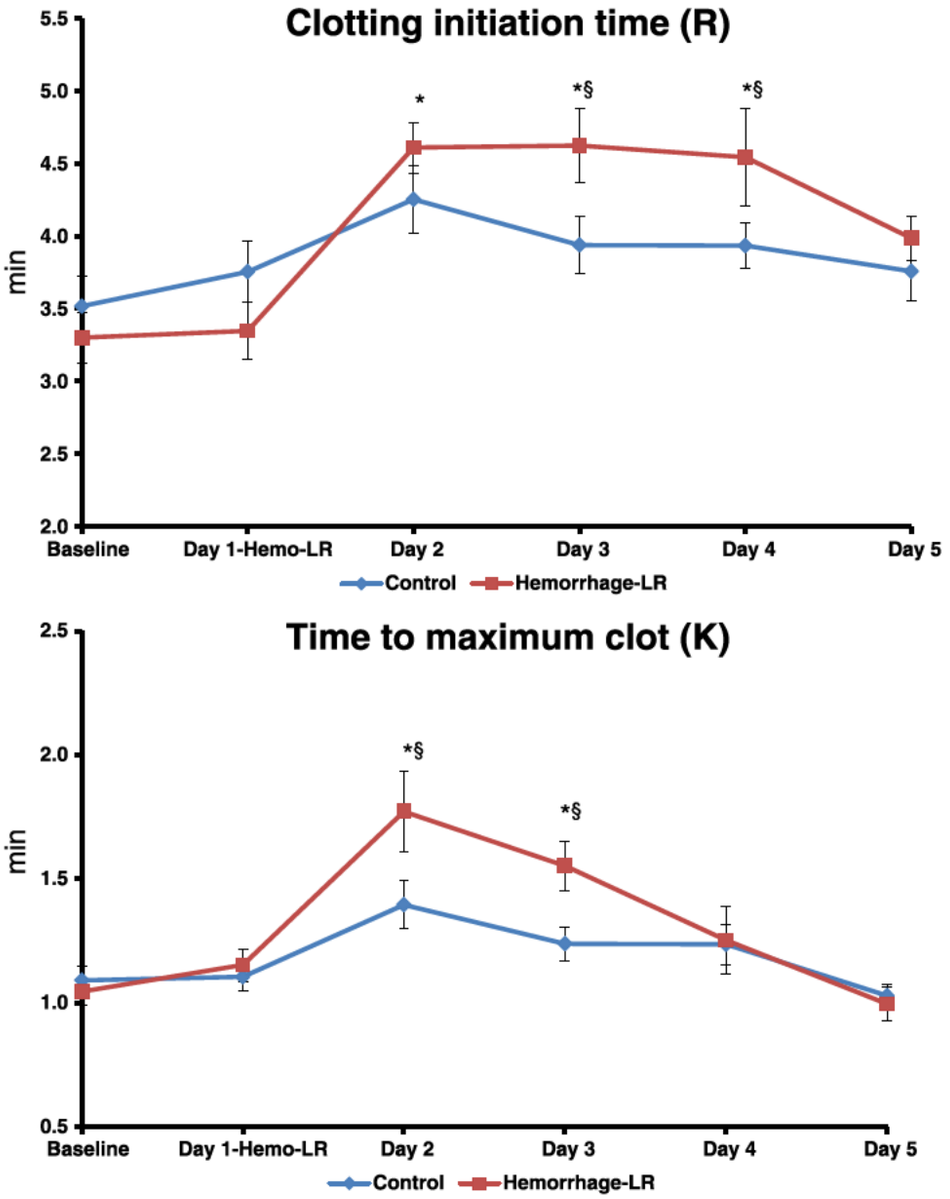

Figure 2. Changes in clotting initiation time (R) and time to maximum clot (K) from thromboelastography measurements after hemorrhage and lactated Ringer's (LR) resuscitation in pigs. Data are represented as mean ± SEM for seven animals per group. *$p < 0.05$ compared with day 1 BL within the group. §$p < 0.05$ compared with corresponding control values.

pig study is possibly due to the moderate severity of hemorrhage used in the study. We are currently investigating severe hemorrhagic shock to correlate changes in coagulation factor levels and coagulation tests.

Clot strength (MA in TEG) represents the contributions of fibrinogen and platelets to the strength of fibrin clots. The increase of clot strength from fibrinogen supplementation was associated with reduction of 24-hour postoperative bleeding in patients undergoing aortic valve operation and ascending aorta replacement,[19] indicating the beneficial effect of improved clot strength under bleeding situations. Maintaining clot strength has been associated with reduced transfusion requirements in trauma patients.[20] In this study, we observed a parallel changing pattern between fibrinogen concentration and clot strength. Fibrinogen concentration was decreased on day 1 after hemorrhage and resuscitation, increased to twice the prehemorrhage level on day 2, and remained at the increased level through day 5. Clot strength was decreased on day 1 after hemorrhage and resuscitation, returned to the prehemorrhage value on day 2, and remained at the BL on days 3, 4, and 5. This parallel profile suggests that clot strength is closely related to fibrinogen availability. Because platelet count and other coagulation factors remained reduced on day 2, the return of clot strength on day 2 suggests a compensatory role of fibrinogen on clot strength. Consistently,

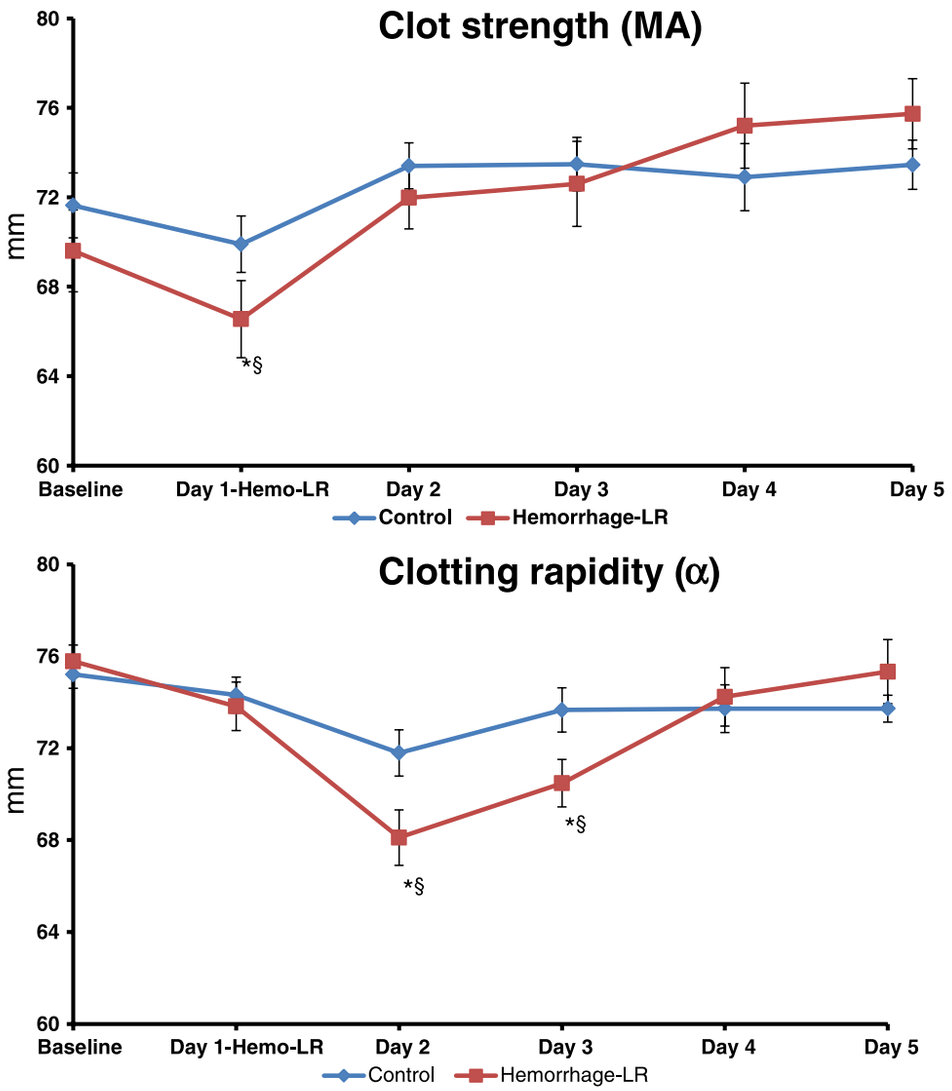

Figure 3. Changes in clot strength (MA) and clotting rapidity (α) from thromboelastography measurements after hemorrhage and lactated Ringer's (LR) resuscitation in pigs. Data are represented as mean ± SEM for seven animals per group. *$p < 0.05$ compared with day 1 BL within the group. §$p < 0.05$ compared with corresponding control values.

the compensatory role of fibrinogen has been suggested recently in other in vitro and in vivo studies. Li et al.[21] have shown that the effect of platelet-blocking substances can be antagonized by increasing fibrinogen concentrations. In pigs with induced thrombocytopenia (platelet counts $< 30 \times 10^3/mm^3$), Velik-Salchner et al.[22] reported that fibrinogen supplementation improved clot firmness (MCF) by rotation thromboelastometry (ROTEM, Tem International GmbH, Munich, Germany) and reduced blood loss after liver injury.

Factor XIII plays important roles in cross-linking and stabilizing fibrinogen polymers[23] and increasing resistance to fibrinolysis.[24] The effects of factor XIII and fibrinogen on clotting function have been investigated in an in vitro study.[25] When human blood samples were diluted by 60% using LR solution, Haas et al. reported that MCF was decreased below the BL of undiluted samples and was improved to the BL by addition of high dose of fibrinogen (equivalent to 120 mg/kg body weight [BW]). Addition of factor XIII (equivalent to 5,000 intrauterine in 70 kg BW) alone, low dose of fibrinogen (equivalent to 60 mg/kg BW) alone, or the combination of factor XIII and low-dose fibrinogen did not improve the decreased MCF. Thus, there might be a required threshold of factor XIII for improving clotting function, although the threshold is unclear at present. In this study, we observed decreases of all coagulation factors after hemorrhage and LR resuscitation and factor XIII did not return to prehemorrhage values at the end of the study on day 5, contrary to other coagulation factors. Despite the lack of recovery of factor XIII, the increased fibrinogen levels on day 2 and thereafter returned clot strength to prehemorrhage values on day 2 and thereafter. These data suggest that the high level of fibrinogen may compensate for the decrease of factor XIII on restoring clot strength in vivo.

The different recovery patterns of coagulation components observed in this study provide useful information for restoration of hemostasis after hemorrhagic shock. Data from this study show that clot strength was compromised within hours after hemorrhage possibly because of reduced fibrinogen availability. When fibrinogen availability was recovered and increased, clot strength was restored at the time when platelet count and other coagulation factors remained at reduced levels. Thus, early supplementation of fibrinogen might be beneficial to restore clot strength in acutely injured patients with bleeding problems.

In summary, we investigated the effects of hemorrhage and LR resuscitation on coagulation in a swine model over a 5-day period. A moderate hemorrhagic shock and LR resuscitation reduced fibrinogen concentration, platelet counts, coagulation factors, and thrombin generation and compromised coagulation function. The recovery patterns among individual coagulation components after hemorrhagic shock were different, with fast recovery in fibrinogen and slow recoveries in platelets and factor XIII. These findings provide useful information for future studies in trauma patients with hemorrhagic shock.

AUTHORSHIP

W.M. designed the study, performed the study with D.C. and other team members, analyzed the data with assistance from statistician Dr. James Aden and Mr. John Jones, and wrote the manuscript. M.D. and L.B. edited the manuscript.

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DISCLOSURE

The authors declare no conflicts of interest.

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