Does Bicarbonate Correct Coagulation Function Impaired by Acidosis in Swine?

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Background: **Coagulopathy is an important contributor to morbidity and mortality in trauma patients. Acidosis contributes to coagulopathy. Acidosis can be neutralized with intravascular bicarbonate, but it is unclear if the coagulation defect is rapidly reversed. The effects of acidosis and bicarbonate neutralization on coagulation function were investigated in vivo.**

Methods: **Acidosis was induced in 12 pigs by infusing 0.2 mol/L HCl to pH 7.1. Pigs were then infused with either LR to maintain a pH of 7.1 (A-LR, n = 6) or 0.3 mol/L bicarbonate to a pH of 7.4 (A-Bi, n = 6). Blood samples were taken at baseline, 15 minutes after acidosis induction, and 15 minutes after bicarbonate neutralization. Coagulation function was assessed by prothrombin time (PT), partial thromboplastin time (PTT), thrombin generation, initial clot formation time (R), clotting rapidity (α), and clot strength (MA).**

Results: **Compared with baseline values, acidosis reduced fibrinogen concentration to 66% ± 2% in A-LR and to 71% ± 3% in A-Bi, and decreased platelet counts to 49% ± 4% in A-LR and to 53% ± 4% in A-Bi. Thrombin generation decreased to 60% ± 4% in A-LR and to 53% ± 7% in A-Bi. Acidosis prolonged PT and PTT about 20% and decreased α and MA. After pH neutralization, fibrinogen and platelet levels remained depleted and no reversal of acidosis-induced changes in thrombin generation, PT, PTT, α, and MA were observed.**

Conclusion: **Acidosis impaired coagulation by depleting fibrinogen and platelets and by inhibiting clotting kinetics. The deficit associated with acidosis was not reversed with bicarbonate pH neutralization.**

Key Words: **Trauma, Fibrinogen, Platelets, Thromboelastography, Thrombin generation.**

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Acidosis in trauma patients is associated with high mortality.[1–4] Common causes for acidosis include tissue hypoxia from massive hemorrhage and massive transfusion of stored blood. A major consequence of acidosis is a disruption in coagulation. Prolonged prothrombin time (PT) and partial thromboplastin time (PTT) have been shown in acidotic trauma patients.[1,4] In a study of 58 massively transfused patients with Injury Severity Scores (ISS) of 30, Cosgriff et al. reported that the probability of developing coagulopathy due to acidosis alone was 58%.[1] When combined with hypothermia and low systolic pressure (<70 mm Hg), the probability rose to 98%.[1] Thus, correcting acidosis-induced coagulopathy represents a major therapeutic goal in saving the lives of trauma patients.

Hypothermia disrupts clotting by slowing coagulation enzyme activity and platelet function. A prolonged PT has been shown in hypothermic patients, experimental animals, and plasma samples chilled in vitro.[5–16] Upon rewarming, correction of enzyme activities, clotting time, and platelet function has been reported.[17–20] Thus, hypothermia reversal may correct hypothermia-associated coagulation defects. However, it is not clear whether the effects of acidosis on coagulation can be similarly reversed through pH neutralization.

The essence of the coagulation process is the formation of fibrin clot from fibrinogen. Thrombin plays a central role in the process, activating platelets and catalyzing the conversion of fibrinogen to fibrin clot.[21] Thrombin generation is essential for rapid and effective clot formation. Thrombin generation consists of initiation and propagation phases.[21] In the initiation phase, factor X is activated and a small amount of thrombin is produced as a result of the formation of the tissue factor activated factor VIIa complex. In the propagation phase, the initial thrombin activates platelets as well as a series of other procoagulant enzymes, resulting in the exponential thrombin burst that is required for normal hemostasis. Thus, changes in thrombin generation kinetics may reveal mechanisms contributing to changes in coagulation function under various circumstances. We recently reported detrimental effects of acidosis and hypothermia on thrombin generation kinetics in a swine model.[22] It is not clear whether acidosis inhibitory effects on thrombin generation can be corrected by pH neutralization.

This study was designed to investigate the mechanisms underlying coagulation changes prompted by acidosis and to assess the immediate efficacy of pH neutralization with sodium bicarbonate in restoring normal coagulation properties. Acidosis of pH 7.1 was induced in swine by infusing 0.2 N HCl,[22] followed by pH neutralization with sodium bicarbonate.

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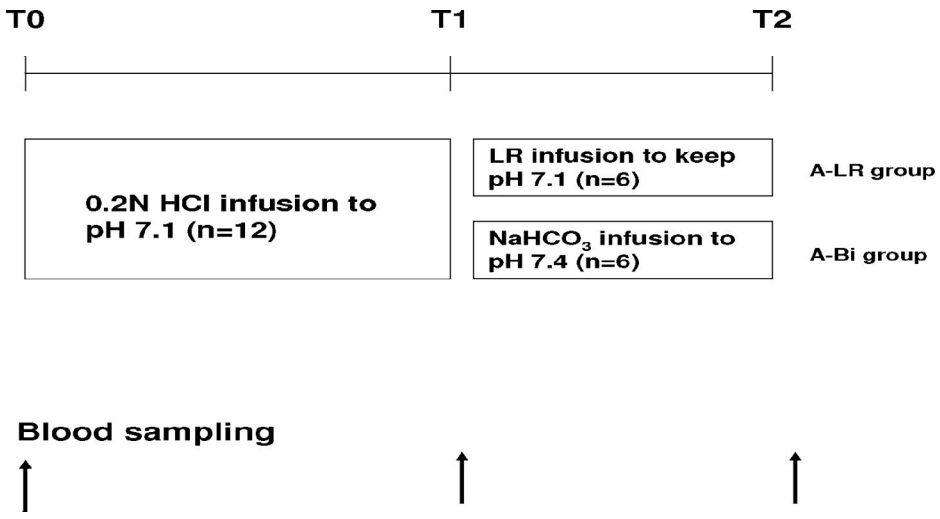

Fig. 1. *Study protocol.*

Changes in coagulation substrates, function, and thrombin generation were evaluated under acidosis and after pharmacologic pH neutralization.

MATERIALS AND METHODS

The experimental studies described in this report were reviewed and approved by the Institutional Animal Care and Use Committee. In conducting the research described here, the authors adhered to the "Guide for the Care and Use of Laboratory Animals," National Research Council, 1996.

Twelve crossbred Yorkshire swine (33 ± 1 kg) obtained from a local vendor were used in this study. After an overnight fast, animals were preanesthetized with glycopyrrolate (0.1 mg/kg) and Telazol (6 mg/kg). Surgical anesthesia was induced with 5% isoflurane in 100% oxygen by mask and then maintained using 1% to 3% isoflurane after intubations. The right femoral artery and the right external jugular vein were cannulated for blood sampling and fluid infusion, respectively. Arterial blood temperature and pH were monitored continuously using intra-arterial sensors precalibrated according to the manufacturer's instructions (Paratrend 7 Trendcare system, Diametrics Medical Inc, Roseville, MN), and placed via a 20-gauge carotid artery cannula. Arterial blood pressure and heart rate were also monitored using an ex vivo pressure transducer connected to the same cannula during the study.

Upon the completion of catheter cannulations and a 15-minute stabilization period, baseline (T0) blood samples were taken for biochemical and coagulation analyses. In the 12 animals, acidosis was induced by infusion of 0.2 mol/L HCl in lactated Ringer's (LR) solution, following the procedures we described previously with modifications in infusion rates.[22] Briefly, a step-increase infusion regimen was used to minimize complications. The infusion rate started at 0.07 mL/kg/min for the initial 60-mL solution, followed by an increase to 0.14 mL/kg/min to reach pH 7.3, another increase in rate to 0.21 mL/kg/min to reach pH 7.25, and a final increase to 0.28 mL/kg/min to reach pH 7.1. Afterward, the 12 pigs were randomized into two groups: the acidosis-bicarbonate group (A-Bi, n = 6) and the acidosis-lactated Ringer's group (A-LR, n = 6). In the A-Bi group, animals were infused with 0.3 mol/L sodium bicarbonate at the rate of 0.28 mL/kg/min to neutralize arterial pH to 7.4. When the target pH of 7.4 was stable for 15 minutes, blood samples were taken in the A-Bi group (T2). Pigs in the A-LR group were infused with LR solution at the same rate and duration as the 0.3 mol/L sodium bicarbonate infusion, before T2 samples were taken. The acidosis study protocol is shown in Fig. 1.

To assess hemodilutional effects on coagulation, six additional animals (from the same vendor and with similar body weights) were infused with LR at rates to mimic the infusion pattern of 0.2N HCl fluid in the 12 acidosis animals described above. This group served as a dilution control.

To minimize coagulation effects from shear-induced platelet activation, all blood samples were collected by inserting a 25-mm single-use catheter made from Tygon tubing (Saint-Gobain Performance Plastics, Akron, OH) into the self-sealing port of the femoral catheter introducer. Blood was gently withdrawn from the catheter and the first 3 mL of blood withdrawn were discarded at each sampling time.

Hematocrit (Hct) and platelet count were measured from citrated blood using an ABX Pentra 120 hematology analyzer (ABX Diagnostics, Inc., Irvine, CA). Blood chemistry was measured using a Dimension Clinical Chemistry System (Dade Behring Inc., Newark, DE). PT and PTT were measured from citrated plasma using BCS Coagulation System (Dade Behring, Deerfield, IL). Plasma fibrinogen concentration was determined by BCS Coagulation System based on fibrinogen functional activities in the clotting process. Activated clotting time (ACT) was determined in fresh whole blood using HRFTCA 510 Hemochron (International Technique Corp, Edison, NJ).

Table 1 Changes in Arterial pH, HCO_3^-, and Electrolytes Under Acidosis and Following Sodium Bicarbonate Neutralization of pH

	Acidosis-Lactated Ringer			Acidosis-Bicarbonate		
	T0	T1	T2	T0	T1	T2
pH	7.40 ± 0.01	7.11 ± 0.01*	7.14 ± 0.02*	7.39 ± 0.01	7.12 ± 0.01*	7.39 ± 0.01†
Na^+ (mM)	139 ± 1	138 ± 1	137 ± 1	141 ± 2	141 ± 3	148 ± 3*,†
K^+ (mM)	4.7 ± 0.1	6.3 ± 0.3*	6.8 ± 0.3*	4.5 ± 0.1	6.7 ± 0.4*	6.3 ± 0.2*
Ca^{++} (mg/dL)	10.0 ± 0.1	9.5 ± 0.0*	9.2 ± 0.1*	10.1 ± 0.2	9.3 ± 0.3*	9.1 ± 0.2*
Cl^- (mM)	98 ± 1	113 ± 1*	110 ± 1*	103 ± 2	115 ± 2*	110 ± 3*
HCO_3^- (mM)	32.3 ± 0.7	16.4 ± 1.1*	19.1 ± 0.7*	33.1 ± 0.6	18.1 ± 1.6*	35.7 ± 2.8†

Data are means ± SE of six animals/group.
*$p < 0.05$ compared to T0 values.
†$p < 0.05$ compared to T1 values.

The coagulation profiles were determined for fresh whole blood with pig thromboplastin using thrombelastography (TEG 5000 Hemostasis Analyzer, Hemoscope Corp, Niles, IL) as previously described.[23] In the TEG measurements, reaction time (R time) is the latency time for initial clot formation. Angle (α) measures the rapidity of fibrin build up and cross-linking. Maximum amplitude (MA) represents maximum strength or stiffness of the clot and CL_{30} indicates the percent of clot lysis at 30 minutes after maximum amplitude is achieved.

Thrombin generation was assessed by measuring thrombin-antithrombin III (TAT) complex from fresh whole blood samples, following the procedure described by Rand et al.[24] Briefly, fresh whole blood samples were aliquoted into 12 tubes and a "quench" solution (50 mmol/L EDTA and 10 mmol/L benzamidine in HEPES-buffered saline) was added to each of the aliquots at different time intervals to stop clot formation. The quenched samples were centrifuged and supernatants were collected for TAT concentration measurement using commercially available ELISA kits (Enzygnost TAT, Dade Behring Inc, Deerfield, IL). The TAT concentrations from the supernatant samples reflect thrombin content generated from fresh whole blood samples before quench time points. This technique has been used to assess thrombin generation kinetics in various studies.[22,25,26]

Statistical Analysis

All results are expressed as means ± SE. Comparisons over time in hemodynamics, substrate concentrations, and clotting measurements between the A-LR and A-Bi groups were made using a mixed model ANOVA with SAS statistical analysis program (Version 8.1, SAS Institute Inc., 1999). Comparisons in thrombin generation over time between the A-LR and A-Bi groups were also made using a mixed model ANOVA. Statistical significance was set at the 0.05 level.

RESULTS

Acidosis Induction and Effects on Coagulation (T0 to T1)

Acidosis of pH 7.1 was successfully induced in all 12 animals in 235 ± 34 minutes (same in both groups), and no animal died as a result of the acidosis. Similar volumes of 0.2N HCl solution were infused to achieve the target pH of 7.1 in A-LR (43 ± 6 mL/kg) and A-Bi (38 ± 4 mL/kg) groups. Similar changes in hemodynamics, metabolic, and coagulation parameters were observed in both groups during the acidosis induction. Mean arterial pressure (MAP) decreased from 105 ± 6 to 76 ± 6 mm Hg in A-LR and from 101 ± 6 to 78 ± 7 mm Hg in A-Bi ($p < 0.05$ from baseline). Heart rate decreased from 99 ± 5 to 83 ± 5 bpm in A-LR and 103 ± 6 to 84 ± 6 bpm in A-Bi ($p < 0.05$). Hct decreased from 31 ± 1% to 28 ± 1% in A-LR and from 29 ± 1% to 25 ± 1% in A-Bi ($p < 0.05$). Arterial base excess (BE) dropped from 7.1 ± 0.7 mmol/L to −12.2 ± 0.9 mmol/L in A-LR and from 7.5 ± 0.6 mmol/L to −10.3 ± 1.5 mmol/L in A-Bi ($p < 0.05$). Concentrations of HCO_3^-, Cl^-, and K^+ increased significantly, while Ca^{++} was decreased by the induction of acidosis (Table 1). There were no significant changes in Na^+, pCO_2, and pO_2 during the acidosis induction.

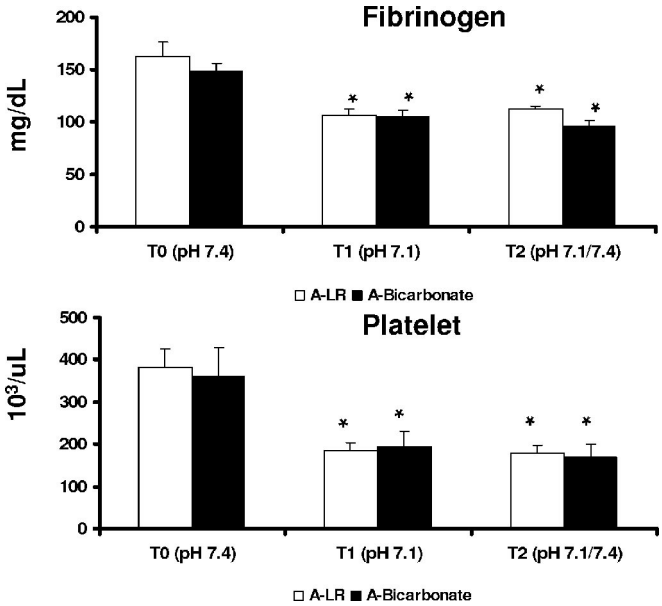

Fig. 2. *Changes in fibrinogen concentration and platelet count during acidosis induction (T1) and bicarbonate neutralization (T2). *p < 0.05, compared with T0.*

Table 2 **Changes in PT, PTT and ACT After Acidosis and Bicarbonate Neutralization of pH**

	Acidosis-Lactated Ringer			Acidosis-Bicarbonate		
	T0	T1	T2	T0	T1	T2
PT (sec)	10 ± 0	12 ± 0*	12 ± 0*	10 ± 0	12 ± 0*	12 ± 0*
PTT (sec)	16 ± 0	20 ± 1*	20 ± 1*	16 ± 0	19 ± 1*	22 ± 1*
ACT (sec)	106 ± 2	130 ± 6*	134 ± 4*	107 ± 6	135 ± 6*	142 ± 11*

Data are means ± SE of six animals/group.
*$p < 0.05$ compared to T0 values.
ACT, activated clotting time.

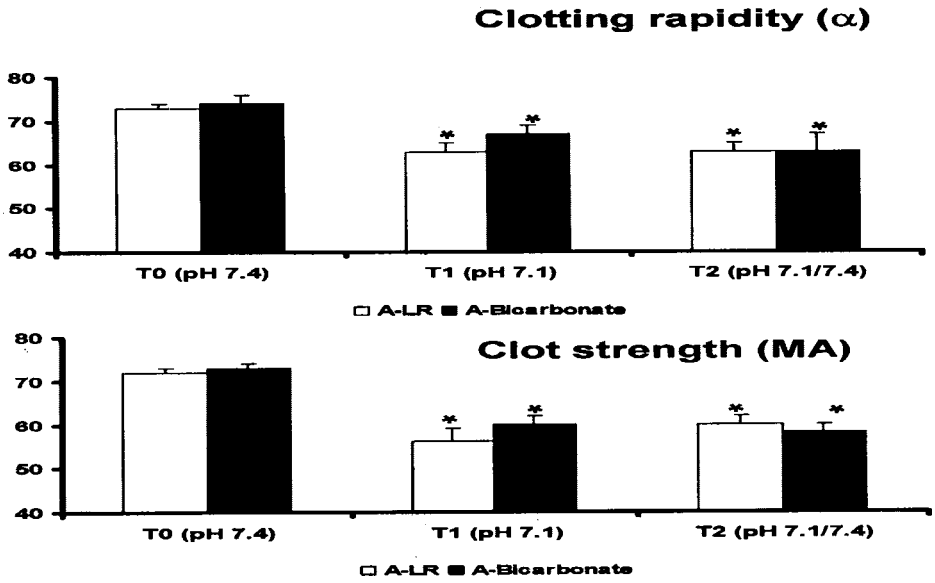

Fig. 3. *Changes in clotting rapidity (α) and lot strength (MA) during acidosis induction (T1) and bicarbonate neutralization (T2).* *p < *0.05, compare to T0.*

Acidosis caused a significant depletion in both fibrinogen concentration and platelet count. Compared with baseline values (T0), fibrinogen concentration dropped to 66 ± 2% in A-LR and 71 ± 3% in A-Bi ($p < 0.05$, Fig 2). Platelet counts were reduced to 49 ± 4% of the baseline values in A-LR and to 53 ± 4% in A-Bi ($p < 0.05$, Fig. 2). There was less depletion in plasma total protein content and albumin content. Plasma total protein concentration decreased to 91 ± 2% of baseline values in A-LR ($p < 0.05$) and 87 ± 2% of baseline values in A-Bi ($p < 0.05$). Serum albumin concentration decreased to 90 ± 3% of baseline values in both A-LR and A-Bi groups ($p < 0.05$).

Acidosis caused significant changes in coagulation function in both groups. PT, PTT, and ACT were consistently prolonged by acidosis in both groups (Table 2). In TEG, clotting rapidity (α) and clot strength (MA) were significantly impaired by acidosis in both groups (Fig. 3). However, the initial clotting time R and fibrinolysis CL_{30} were not changed by acidosis in either group.

There were no differences in thrombin generation kinetics between the two groups at baseline (Fig 4, T0). Following acidosis induction (T1), similar changing patterns in thrombin generation were observed in the two groups (Fig. 4, T1). The onset of thrombin generation (in the initiation phase) remained unchanged in both groups. However, thrombin generation afterward (in the propagation phase) was severely impaired by acidosis in both groups (Fig. 4, T1). Compared with baseline thrombin generation, acidosis decreased thrombin generation to 60 ± 4%, 54 ± 4%, 57 ± 3% and 66 ± 6% in A-LR group, and to 53 ± 7%, 52 ± 9%, 53 ± 8% and 56 ± 10% in A-Bi group, at 7 minutes, 10 minutes, 15 minutes, and 20 minutes quench time points, respectively (all $p < 0.05$).

In the volume control group, the infusion of 1.5 L of LR over 235 minutes to mimic acidosis induction in the A-LR and A-Bi groups did not change fibrinogen concentration (122.9 ± 3.3 mg/dL at T0 and 126.3 ± 5.8 mg/dL at T1) or platelet count (342.5 ± 28.2 10^3/mL at T0 and 315.0 ± 36.4 10^3/mL at T1). Similarly, there were no changes in initial clotting time R (3.1 ± 0.1 at T0 and 3.1 ± 0.2 at T1), clotting rapidity α (74.3 ± 0.3 at T0 and 74.5 ± 0.3 at T1), clot strength MA (70.8 ± 0.8 at T0 and 69.9 ± 0.7 at T1), PT (10.2 ± 0.2 s at T0 and 9.8 ± 0.1 s at T1), PTT (16.1 ± 0.1 s at T0 and 16.6 ± 0.3 s at T1), or ACT (103 ± 6 s at T0 and 100 ± 2 s at T1). Thus, the hemodilutional effect on coagulation from acidosis induction is minimal.

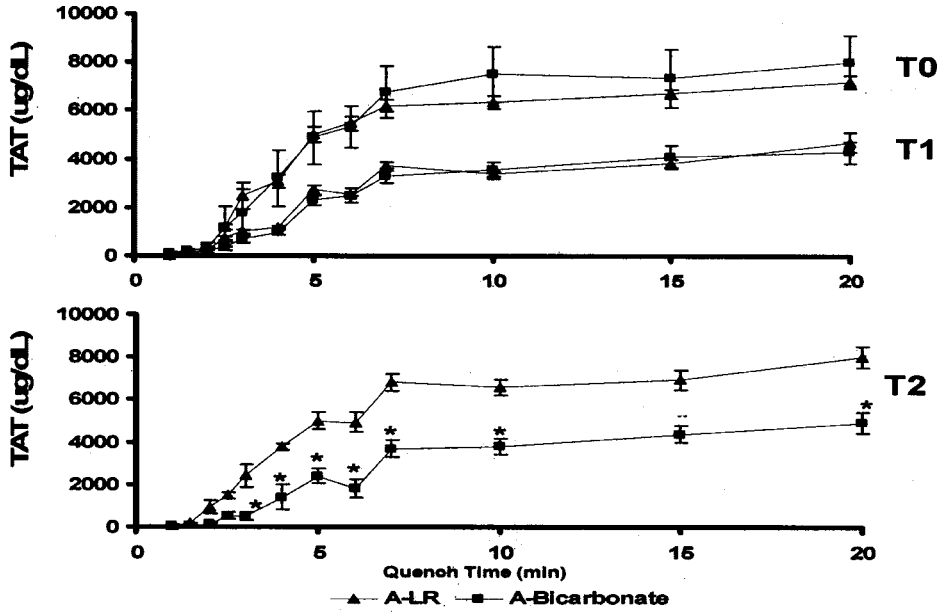

Fig. 4. *Thrombin generation kinetics at baseline (T0, pH 7.4), acidosis (T1, pH 7.1), and after bicarbonate neutralization (T2 pH 7.4, or without neutralization pH 7.1) during the study. *p $<$ 0.05, A-LR versus A-Bi at T2.*

Bicarbonate pH Neutralization (T1 to T2)

Bicarbonate infusion in the A-Bi group (26 ± 3 mL/kg) raised the arterial pH from 7.1 (T1) to 7.4 (T2) in 69 ± 19 minutes. The sodium bicarbonate infusion raised HCO_3^- concentration from 18.1 ± 1.6 mmol/L to 35.7 ± 2.8 mmol/L ($p < 0.05$) and Na^+ from 140.7 ± 2.7 mmol/L to 148.3 ± 3.2 mmol/L (Table 1; $p < 0.05$). The deficit in BE was reversed from –10.3 ± 1.5 mmol/L to 10.2 ± 2.6 mmol/L. In the A-LR group, arterial pH remained at 7.1 after LR infusion (25 ± 4 mL/kg). During the LR infusion, bicarbonate concentrations in the A-LR increased from 16.4 ± 1.2 mmol/L to 19.1 ± 0.7 mmol/L, with no change in Na^+ concentrations (Table 1). BE in the A-LR animals remained negative (–12.2 ± 0.9 mmol/L at T1 and –8.3 ± 0.4 mmol/L at T2). There were no changes observed in Hct, pCO_2, and pO_2 between T1 and T2 in either group (data not shown).

Although bicarbonate infusion successfully reversed pH and BE in the A-Bi group, fibrinogen concentration and platelet count remained unchanged during pH neutralization (T2 versus T1; Fig. 2). There were also no significant changes in fibrinogen and platelet levels in the A-LR group at T2 compared with T1 (Fig. 2). Plasma total protein and albumin concentrations remained unchanged between T1 and T2 in both groups. In addition, bicarbonate neutralization of arterial pH did not significantly affect coagulation function at T2 compared with T1. Clotting rapidity (α) and clot strength (MA) remained at impaired levels in both groups (Fig. 3). PT, PTT, and activated clotting time remained unchanged after bicarbonate or LR infusion (Table 2).

Thrombin generation kinetics following pH neutralization (the A-Bi group) was compared with that without pH correction (the A-LR group) at T2 in Figure 4. The onset of thrombin generation remained unchanged in both groups. In the A-LR group while pH was maintained at 7.1, thrombin generation at the end of the study (T2) returned to values similar to baseline values in the same group (T0), indicating a possible recovery of thrombin generation (Fig. 4). However, this recovery was not shown in the animals after bicarbonate pH neutralization. The thrombin generation after bicarbonate pH neutralization (T2) in the A-Bi group was similar to the impaired values following acidosis induction (T1) in the same group (Fig. 4). At 7 minutes, 10 minutes, 15 minutes, and 20 minutes quench time points, thrombin generation in the A-Bi group was 55 ± 7%, 59 ± 8%, 64 ± 6%, and 62 ± 6% of the values in the pH uncorrected A-LR group (Fig. 4, T2, all $p < 0.05$).

DISCUSSION

We investigated alterations and underlying mechanisms in the coagulation process under acidosis and following arterial pH neutralization with bicarbonate in a swine model. Our data demonstrate that acidosis is detrimental to the coagulation process: causing approximate 35% depletion in fibrinogen concentration, 50% decrease in platelet count, and 50% inhibition in thrombin generation in both groups. Consequently, blood clotting time was prolonged and clot formation and strength were impaired. Infusion of bicarbonate neutralized pH to 7.4; however, it did not immediately reverse any of the detrimental changes caused by acidosis. The lack of improvement with bicarbonate neutralization indicates the complexity and challenge in treating acidosis coagulopathy.

Fibrinogen is the precursor protein in the coagulation process. Proper clot formation requires sufficient fibrinogen available in the circulation. At any time, fibrinogen availabil-

ity reflects the dynamic balance of production and consumption. In this study, acidosis of pH 7.1 was induced by infusion 0.2N HCl solution over nearly a 4-hour period. Fibrinogen concentration (measured by fibrinogen functional activity) was decreased by about 35% after achievement of the target level of acidosis. Because plasma total protein was only decreased about 10% to 15% during the infusion, it is possible that changes in fibrinogen metabolism, such as accelerated degradation and/or inhibited synthesis, may contribute to the concentration decrease. Because fibrinogen synthesis rate in normal pigs is about 2% to 4% per hour,[27,28] the 35% depletion of fibrinogen appears to be likely due to accelerated fibrinogen degradation, rather than inhibited synthesis. In fact, an accelerated degradation with unchanged synthesis in fibrinogen has been shown in pigs following a moderate hemorrhage.[27] Although changes in fibrinogen degradation following acidosis are yet to be defined, it is clear that the fibrinogen loss by acidosis is irreversible, because bicarbonate neutralization of arterial pH did not reverse the changes in fibrinogen levels. Thus, without exogenous intervention, it would take over 10 hours for endogenous synthesis to replenish fibrinogen availability to normalize coagulation following acidosis, even if there were no degradation. This notion is supported by a recent clinical observation.[29] In a prospective study of 202 major torso trauma patients with significant coagulopathy at ICU admission, McKinley et al. reported that coagulopathy remained uncorrected throughout a 24-hour ICU period, despite successful efforts in normalizing acidosis.[29] Taken together our data support the notion of a possible need for fibrinogen supplementation in treating acute trauma patients with acidosis coagulopathy.

We also assessed changes in thrombin generation kinetics induced by acidosis and following bicarbonate neutralization of arterial pH. Our data showed that the initial thrombin generation remained unchanged under acidosis and after bicarbonate pH neutralization, indicating that changes in pH have minimal effects on the initiation phase of thrombin generation process. However, thrombin generation in the propagation phase was inhibited by acidosis by as much as 50%. Acidosis appears to affect different pathways in the coagulation process in different ways. Meanwhile, we observed an unexpected recovery of thrombin generation from the 50% inhibition to baseline values in the pH-uncorrected group at the end of the study. However, this recovery in thrombin generation was not accompanied by a recovery in coagulation function, suggesting that correction of substrate depletion might also be necessary for normalizing coagulation. In addition, the recovery in thrombin generation, however, was not present in animals with bicarbonate pH neutralization. The 50% inhibition in thrombin generation by acidosis remained unchanged after bicarbonate pH neutralization. Because there are many enzymes, factors, and cofactors involved in the thrombin generation propagation phase in comparison with the initiation phase (tissue factor and factor VII), we are unable to define specific sites contributing to the 50% inhibition at present. Neither can we explain the recovery of thrombin generation in the pH-uncorrected group nor its continued inhibition on thrombin generation with bicarbonate pH correction. Similarly, we are unable to explain the 50% decrease in platelet count by acidosis. Additional studies are needed to clarify the mechanisms responsible for the drop of platelet count and potential functional changes.

In this study, a comprehensive approach was used to assess the effects of acidosis and arterial pH neutralization with bicarbonate on coagulation. Our measurements included TEG, PT, PTT, ACT, in combination with substrate (fibrinogen) concentration and thrombin generation kinetics. TEG provides an overview of the blood clotting profile. PT and PTT provide general measures of intrinsic common pathway integrity and extrinsic common pathway integrity, respectively. Activated clotting time (ACT) assesses overall clotting properties from fresh whole blood. Consistent and complementary results were observed in this study. For example, the initial clotting time (R) in TEG was not changed in either group throughout the study, which coincided with unchanged initial thrombin generation throughout the study in both groups. Maximum amplitude (MA) represents clot strength, which is affected by platelet and fibrinogen levels. Clotting rapidity (α) measures fibrin build up and crosslinking, which is induced by thrombin generation. In this study, we observed decreases in both MA and α following acidosis, which agreed well with depletions in fibrinogen level, platelet counts, and impairments in thrombin generation. Further, PT, PTT, and ACT were similarly prolonged by acidosis, and remained prolonged after pH neutralization with bicarbonate.

Although sodium bicarbonate has variously been part of clinical practice in treating acute lactic acidosis,[30,31] there is limited information available about coagulation changes following bicarbonate administration. Wong et al. reported that sodium bicarbonate interfered with the conversion of fibrinogen to fibrin.[32,33] By adding sodium bicarbonate or potassium bicarbonate to blood samples, these investigators showed that the thrombin time increased by 20% when the bicarbonate concentration was increased from 12 mEq/L blood to 37 mEq/L.[32] In the same report, when bicarbonate was infused in acidotic patients (n = 4), a 50% to 100% prolongation in PT and PTT occurred.[32] Combining these reports with our findings, it is possible that bicarbonate interferes with fibrin monomer assembly into fibrin polymer and inhibits thrombin production. Although the mechanisms remain to be clarified, bicarbonate may not be the best choice of agents to neutralize pH and reverse coagulopathy in acidosis patients.

As acute phase proteins, both fibrinogen and albumin are essential liver proteins with specific metabolic function and regulations. Changes in their levels in the circulation can potentially impact physiologic function. As the precursor in the coagulation process, fibrinogen is primarily involved in maintaining hemostasis. With a molecular weight of 340,000, fibrinogen is primarily confined in the vascular pool and has

a half life of 3 to 4 days.[34] Albumin, as a transport protein, has a molecular weight of 65,000 and less than 50% of its amount is confined in the vascular pool. The half-life of albumin is 17 to 18 days.[34] In this study, we observed a 35% drop in fibrinogen concentration by acidosis, while there was only about a 10% decrease in albumin concentration. These data suggest that the metabolic effects of acidosis may vary with different proteins. It is also possible that following acidosis insult, some of the albumin loss from the circulation was compensated for by albumin refilling from the extravascular to the intravascular pool. Whatever mechanisms are involved, it is clear that fibrinogen and the coagulation process are highly vulnerable to acidosis.

In this study, acidosis was induced by infusing 1.5 L of 0.2N HCl in LR for 4 hours. The possible hemodilutional effects related to the volume infusion were assessed in the volume control group. Because there were no changes observed in fibrinogen concentration, platelet counts, and coagulation function in the group, it is clear that the hemodilutional effects on coagulation from the infusion of acid was minimal compared with the effects of acidosis itself. In the second portion of this experiment, a smaller volume (0.9 L) was infused to study the effects of pH neutralization. This volume infusion did not cause significant changes in plasma total protein, albumin and fibrinogen levels. Further, based on the changes in plasma protein observed in the present study, we estimate a 10% to 15% hemodilution effect of the infused fluid used throughout the entire study. Previous studies suggest that a hemodilution of at least 50% is required before a significant change in clotting parameters is observed,[35–38] it is thus reasonable to conclude that the hemodilutional effect in this study is minimal and that the observed coagulation changes result primarily from acidosis and pH neutralization with bicarbonate.

Both lactic acid and hydrochloric acid have been used to induce intravascular acidosis to investigate acidosis effects on coagulation function.[22,39–41] Because life-threatening acidosis in trauma is often a result of lactic acidosis, we initially tried lactic acid but encountered technical difficulties of hemolysis and high mortality. To overcome these problems, we used hydrochloric acid and completed the study. Although physiologic differences between the two models remain to be clarified, both models result in intravascular pH changes. The primary purpose of this study was to investigate coagulation changes under different intravascular pH. Therefore, we consider the effects observed in this study are reasonably valid to the purpose of the study.

In summary, we investigated possible mechanisms related to coagulation alterations induced by acidosis and the potential for bicarbonate to reverse the altered coagulation function. Acidosis impaired the coagulation process by depleting some coagulation substrates and inhibiting the thrombin generation burst. As a result, clotting time was prolonged and clot strength compromised. Bicarbonate pH neutralization did not immediately reverse any of the coagulation changes, and it actually prevented the return of normal thrombin generation that was observed in pH uncorrected group. Because it appears that sodium bicarbonate adversely affects the recovery of coagulation function, efforts are warranted to search for other pH neutralization methods that will improve acidosis-induced coagulopathy. Further, based on our previous observations on the slow endogenous fibrinogen synthesis,[27] it would appear that simply reversing acidosis with a drug or resuscitation fluid may not be sufficient to restore normal coagulation function. Our results suggest that specific clotting factor replacement may be necessary for the ultimate reversal of impaired coagulation associated with acidosis.

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REFERENCES

1. Cosgriff N, Moore EE, Sauaia A, et al. Predicting life-threatening coagulopathy in the massively transfused trauma patient: hypothermia and acidoses revisited. *J Trauma.* 1997;42:857–861.
2. Mikhail J. The trauma triad of death: hypothermia, acidosis, and coagulopathy. *AACN Clin Issues.* 1999;10:85–94.
3. Ferrara A, MacArthur JD, Wright HK, et al. Hypothermia and acidosis worsen coagulopathy in the patient requiring massive transfusion. *Am J Surg.* 1990;160:515–518.
4. Moore EE. Thomas G. Orr Memorial Lecture. Staged laparotomy for the hypothermia, acidosis, and coagulopathy syndrome. *Am J Surg.* 1996;172:405–410.
5. Jurkovich GJ, Greiser WB, Luterman A, et al. Hypothermia in trauma victims: an ominous predictor of survival. *J Trauma.* 1987; 27:1019–1024.
6. Luna GK, Maier RV, Pavlin EG, et al. Incidence and effect of hypothermia in seriously injured patients. *J Trauma.* 1987;27: 1014–1018.
7. Gregory JS, Flancbaum L, Townsend MC, et al. Incidence and timing of hypothermia in trauma patients undergoing operations. *J Trauma.* 1991;31:795–798.
8. Bernabei AF, Levison MA, Bender JS. The effects of hypothermia and injury severity on blood loss during trauma laparotomy. *J Trauma.* 1992;33:835–839.
9. Britt LD, Dascombe WH, Rodriguez A. New horizons in management of hypothermia and frostbite injury. *Surg Clin North Am.* 1991;71:345–370.
10. Steinemann S, Shackford SR, Davis JW. Implications of admission hypothermia in trauma patients. *J Trauma.* 1990;30:200–202.
11. Staab DB, Sorensen VJ, Fath JJ, et al. Coagulation defects resulting from ambient temperature-induced hypothermia. *J Trauma.* 1994; 36:634–638.
12. Watts DD, Trask A, Soeken K, et al. Hypothermic coagulopathy in trauma: effect of varying levels of hypothermia on enzyme speed, platelet function, and fibrinolytic activity. *J Trauma.* 1998;44: 846–854.

13. Reed RL, 2nd, Bracey AW, Jr., Hudson JD, et al. Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. *Circ Shock.* 1990;32:141–152.
14. Rohrer MJ, Natale AM. Effect of hypothermia on the coagulation cascade. *Crit Care Med.* 1992;20:1402–1405.
15. Kermode JC, Zheng Q, Milner EP. Marked temperature dependence of the platelet calcium signal induced by human von Willebrand factor. *Blood.* 1999;94:199–207.
16. Krurrek MM RR. Effect of hypothermia on enzymatic activity of thrombin and plasmin. *Surgical Forum.* 1987;38:221–223.
17. Valeri CR, Feingold H, Cassidy G, et al. Hypothermia-induced reversible platelet dysfunction. *Ann Surg.* 1987;205:175–181.
18. Yoshihara H, Yamamoto T, Mihara H. Changes in coagulation and fibrinolysis occurring in dogs during hypothermia. *Thromb Res.* 1985;37:503–512.
19. Gentilello LM, Cortes V, Moujaes S, et al. Continuous arteriovenous rewarming: experimental results and thermodynamic model simulation of treatment for hypothermia. *J Trauma.* 1990;30: 1436–1449.
20. Valeri CR, Khabbaz K, Khuri SF, et al. Effect of skin temperature on platelet function in patients undergoing extracorporeal bypass. *J Thorac Cardiovasc Surg.* 1992;104:108–116.
21. Mann KG, Brummel K, Butenas S. What is all that thrombin for? *J Thromb Haemost.* 2003;1:1504–1514.
22. Martini WZ, Pusateri AE, Uscilowicz JM, et al. Independent contributions of hypothermia and acidosis to coagulopathy in swine. *J Trauma.* 2005;58:1002–1009.
23. Pusateri AE, Ryan KL, Delgado AV, et al. Effects of increasing doses of activated recombinant factor VII on haemostatic parameters in swine. *Thromb Haemost.* 2005;93:275–283.
24. Rand MD, Lock JB, van't Veer C et al. Blood clotting in minimally altered whole blood. *Blood.* 1996;88:3432–3445.
25. Butenas S, Brummel KE, Branda RF, et al. Mechanism of factor VIIa-dependent coagulation in hemophilia blood. *Blood.* 2002; 99:923–930.
26. Butenas S, Brummel KE, Bouchard BA, et al. How factor VIIa works in hemophilia. *J Thromb Haemost.* 2003;1:1158–1160.
27. Martini WZ, Chinkes DL, Pusateri AE, et al. Acute changes in fibrinogen metabolism and coagulation after hemorrhage in pigs. *Am J Physiol Endocrinol Metab.* 2005;289:E930–E934.
28. Jahoor F, Wykes L, Del Rosario M, et al. Chronic protein undernutrition and an acute inflammatory stimulus elicit different protein kinetic responses in plasma but not in muscle of piglets. *J Nutr.* 1999;129:693–699.
29. McKinley BA GE, Balldin BC, Majid TH, et al. Revisiting the "bloody vicious cycle." *Shock.* 2004;2:47.
30. Hindman BJ. Sodium bicarbonate in the treatment of subtypes of acute lactic acidosis: physiologic considerations. *Anesthesiology.* 1990;72:1064–1076.
31. Adrogue HJ, Madias NE. Management of life-threatening acid-base disorders. Second of two parts. *N Engl J Med.* 1998;338:107–111.
32. Wong DW, Mishkin FS, Tanaka TT. The effects of bicarbonate on blood coagulation. *JAMA.* 1980;244:61–62.
33. Wong DW. Effect of sodium bicarbonate on in vitro conversion of fibrinogen to fibrin. *J Pharm Sci.* 1980;69:978–980.
34. Fleck A. Plasma proteins as nutritional indicators in the perioperative period. *Br J Clin Pract Suppl.* 1988;63:20–24.
35. DeLoughery TG. Coagulation defects in trauma patients: etiology, recognition, and therapy. *Crit Care Clin.* 2004;20:13–24.
36. Lim RC, Jr., Olcott Ct, Robinson AJ, et al. Platelet response and coagulation changes following massive blood replacement. *J Trauma.* 1973;13:577–582.
37. Wilson RF, Mammen E, Walt AJ. Eight years of experience with massive blood transfusions. *J Trauma.* 1971;11:275–285.
38. Fries D, Krismer A, Klingler A, et al. Effect of fibrinogen on reversal of dilutional coagulopathy: a porcine model. *Br J Anaesth.* 2005;95:172–177.
39. Dunn EL, Moore EE, Breslich DJ, et al. Acidosis-induced coagulopathy. *Surg Forum.* 1979;30:471–473.
40. Hardaway RM, Elovitz MJ, Brewster WR, Jr., et al. Clotting time of heparinized blood. Influence of acidosis. *Arch Surg.* 1964;89: 701–705.
41. Crowell JW, Houston B. Effect of acidity on blood coagulation. *Am J Physiol.* 1961;201:379–382.