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Acute coagulopathy in a porcine venous hemorrhage and ischemia reperfusion model

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Abstract

BACKGROUND: Injury-related coagulopathy is a complex process. We analyzed coagulation in a swine model of shock using rotational thromboelastometry (ROTEM).

METHODS: Forty-eight swine underwent laparotomy, 35% hemorrhage, supraceliac aortic cross-clamp, then reperfusion and resuscitation. ROTEM measurements and standard labs were taken at baseline and 6 hours into resuscitation.

RESULTS: Clot formation time (98 vs 53 seconds, $P = .001$) and international normalized ratio (1.67 vs 1.01, $P < .001$) were prolonged after resuscitation. Maximum clot firmness (61 vs 72 mm, $P < .001$) and fibrinogen levels (94 vs 165, $P < .001$) declined significantly during resuscitation. Despite decreased fibrinogen levels, there was no significant increase in fibrinolysis as measured by maximum lysis (3.9% vs 3.8%, $P = .99$).

CONCLUSIONS: ROTEM demonstrated the development of an acute coagulopathy. The most significant impacts on coagulopathy were seen with clot initiation and fibrin polymerization. Clot strength decreased over time, although there was little impact on clot breakdown because of fibrinolysis.
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Acute coagulopathy is present in 1 in 4 presenting trauma patients and is associated with a 4-fold increase in mortality.[1–4] The Acute Coagulopathy of Trauma (ACOT) is a dynamic process thought to be because of the combined effects of shock, hemodilution, hypothermia, acidemia, and tissue injury. The result is a global disruption of coagulation factors, platelet dysfunction, hyperfibrinolysis, and ultimately a significant risk of mortality.[1]

Because of the acute nature of traumatic injury and the difficulty of prospective randomized trials in the emergent setting, the study of trauma has largely been relegated to retrospective analyses and animal experimentation. New drug therapies to stem ACOT are particularly difficult to study and compare in human trauma, as many of these therapies are only approved for off-label use. For this reason, large animal models of hemorrhagic shock have been developed. Porcine models predominate in trauma research, as swine are similar to humans in terms of their hemodynamic and pulmonary response to injury, hemorrhage, and resuscitation.[5,6]

Because of the many physiologic and genetic similarities between humans and swine, numerous investigators have

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used the swine large animal model to study the response to acute injury and therapeutic interventions.[5–19] Previous studies from our institution have utilized a reproducible porcine model of acute hemorrhage and ischemia–reperfusion to study ACOT.[8,11,14–16] To compare the swine and human experience of acute coagulopathy, a baseline understanding of the porcine coagulation response to injury, which is inherently different from that of a human, must be established. Recent reports have suggested, for example, that hypothermia does not exacerbate an acute traumatic coagulopathy in pigs, which lies in contrast to the human coagulation response.[12] Furthermore, swine are resistant to metabolic acidosis[19] and are hypercoagulable at baseline,[20] both of which challenge our ability to extrapolate the human response to trauma interventions from porcine models.

Although no animal model has accurately recreated all elements of traumatic injury in a human (much less the physiologic response), we believe that our previously described swine hemorrhage and ischemia–reperfusion model serves as a reliable proxy. Tantamount to this claim are the reliable production of profound metabolic acidosis as well as acute coagulopthy. This study retrospectively reviews the results of coagulation studies in the control animals from a complex, multi-arm study using our swine hemorrhage and ischemia–reperfusion model. Post hemorrhage–ischemia–reperfusion coagulation abnormalities were assessed using dynamic thromboelastometry as well as static coagulation parameters.

Methods

Approval of the Madigan Army Medical Center Institutional Animal Care and Use Committee was obtained before initiation of the study. All animals were maintained in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Research Council/Institute of Laboratory Animal Research.

The protocol for our porcine injury–ischemia–reperfusion model has been previously described,[11,14–16] but will be outlined here in brief. Data from all control animals from a complex, multi-arm experiment were obtained and retrospectively reviewed. Forty-eight genetically similar adult Yorkshire swine weighing between 35 and 55 kg were included in the study. All animals were obtained from a local breeder. The protocol is as follows. General anesthesia is induced and a right neck dissection is performed. An arterial line is placed in the right carotid artery and a pulmonary artery catheter is placed via a 9 Fr sheath placed in the external jugular vein.

A midline laparotomy is performed and a urinary bladder catheter is placed. The supraceliac aorta is then dissected out and a vessel loop is placed around it to facilitate quick access. A 6 Fr sheath is then placed in the infrarenal inferior vena cava (IVC), for controlled hemorrhage.

At this point, a 35% hemorrhage is performed by withdrawing 25 mL of blood per kg via the IVC catheter. This is performed over approximately 20 minutes. After a short stabilization period, an atraumatic vascular clamp is used to cross-clamp the supraceliac aorta. Aortic clamping is continued for 50 minutes. At the 45-minute mark an epinephrine infusion is started at 50 mcg/minute. Once 50 minutes of clamping has elapsed, the cross-clamp is removed and digital pressure is held on the aorta. Digital pressure is slowly withdrawn over 5 minutes. The abdomen is then closed with penetrating towel clips.

A 6-hour maintenance and resuscitation phase is then undertaken, after which the pigs are euthanized with a barbiturate-based euthanasia solution injected intravenously. During this phase, mean arterial pressures are maintained between 40 and 50 mmHg via the standardized use of a crystalloid and epinephrine-based protocol.

Consistently obtained variables for analysis included temperature, rotational thromboelastometry (ROTEM) using the Ex-Tem assay, pH, lactate, fibrinogen, and international normalized ratio (INR) at baseline and at the conclusion of resuscitation. Specific ROTEM measurements analyzed included clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), and maximum lysis (ML).

Descriptive and univariate analyses were performed using paired *t* tests. Statistical analysis was performed using PASW Statistics 18.0 (SPSS, Inc, Chicago, IL) and significance was set at $P < .05$.

Results

All 48 animals survived to the 6-hour time point. Baseline values reflected the post-anesthesia, pre-injury physiologic state. Six hours after injury, hemorrhage, and ischemia–reperfusion, the mean temperature, pH, fibrinogen, and MCF were all decreased. Lactate, INR, CT, and CFT increased. Percent ML did not change over time (Table 1). The model successfully replicated the metabolic acidosis seen in polytrauma patients and showed similar changes in coagulation.

Comments

We evaluated the impact of hemorrhage, ischemia–reperfusion, and acidosis on porcine coagulation. We

Table 1 Baseline versus 6-hour physiologic variables

	Baseline	6–Hour resuscitation	*P* value
Temperature	101	100.4	.001
pH	7.56	7.1	<.001
Lactate	1.5	9.7	.01
INR	1.01	1.67	<.001
Fibrinogen	167.6	95.2	.005
CT	50.3	74.8	.001
CFT	53.4	98.1	.001
MCF	72	61	<.001
ML	3.8	3.9	.90

CFT = clot formation time; CT = clotting time; INR = international normalized ratio; MCF = maximum clot firmness; ML = maximum lysis.