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Full Length Article

Differential contributions of platelets and fibrinogen to early coagulopathy in a rat model of hemorrhagic shock

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ABSTRACT

Background: The mechanisms of early traumatic-induced coagulopathy are not well understood. Our aim was to examine the role of platelets and fibrinogen to early coagulopathy in the rat after hemorrhagic shock.
Methods: Adult Sprague-Dawley rats were anesthetized and randomly assigned to: 1) Baseline, 2) Hemorrhage or 3) Shock (n = 10 each). Controlled phlebotomy occurred over 20 min and animals were left in shock 60 min. Coagulation was assessed using PT, aPTT, ROTEM and ELISAs.
Results: PT and aPTT increased 5 to 7 times following hemorrhage and shock. Prolongation of EXTEM and INTEM clotting times, lower clot elasticity and increased EXTEM lysis index (LI) indicated a hypocoagulopathy. After 20 min hemorrhage, LI(30–60) in FIBTEM was ~100%, EXTEM 83–87% and APTEM 80–82% indicating a platelet contribution to the coagulopathy with no hyperfibrinolysis. After 60 min shock, the situation was reversed with fibrinogen loss being a contributor. This apparent switch from a platelet- to a fibrinogen-based coagulopathy, with fibrinolysis, was supported by ≥15% in maximum lysis (ML), a threefold increase in plasma PAI-1 after hemorrhage, and undetectable levels after shock. Curiously, the relative contribution of fibrinogen/platelet ratio to clot amplitude, determined from FIBTEM/EXTEM A10 ratio (and MCF), remained unchanged at ~1:5 for baseline, hemorrhage and shock despite a progressive hypocoagulopathy. Significant increases in P-selectin, acidosis and lactate indicated systemic endothelial damage and tissue hypoperfusion.
Conclusions: Hypocoagulopathy following severe hemorrhage and shock in the rat appeared to involve a two-step process of platelet dysfunction followed by fibrinogen impairment, possibly linked to progressive endothelial dysfunction.

1. Introduction

Hemorrhage continues to be responsible for about one-third of all trauma fatalities globally and coagulopathic bleeding is an independent risk factor for complications and death [1–4]. On the battlefield, hemorrhage accounts for up to 90% of potentially preventable traumatic deaths [5,6]. Early traumatic-induced coagulopathy (TIC) can occur in around 25% of severely traumatized patients [7,8], and other bleeding coagulopathies during obstetric hemorrhage, cardiac arrest, burns, sepsis, and major surgery [9–11].

The ability to detect and treat early TIC requires an accurate assessment of the nature and extent of the hemostatic disorder, including the presence of hyperfibrinolysis [12]. Many advances have been made with the use of thromboelastometry (ROTEM®) and thromboelastography (TEG®) to guide treatment [3,10,13–15]. Despite a strong focus on detecting changes of fibrinogen after trauma [15,16], increasing interest is emerging on the role of platelet dysfunction to TIC, despite sufficient platelet numbers [17–19]. More recently, Kornblith and colleagues showed that platelets exerted a greater contribution to clot strength relative to fibrinogen in coagulopathic trauma patients than previously thought [20]. The topic is clinically important to early resuscitation and restorative hemostasis given the opposing fibrinogen-based European and platelet-based US resuscitation practices [3,20].

The aim of our study was to investigate the relative contributions of platelets and fibrinogen after 20 min controlled bleed, and after 60 min untreated shock, in a rat model. We have previously shown the presence of hypocoagulopathy at 20 min bleed, which worsens after 60 min shock [21–23]. However, we have not examined hyperfibrinolysis at these two different pathological states using the ROTEM tests EXTEM, FIBTEM and APTEM. We hypothesized that the progressive hypocoagulopathy may involve differential platelet and fibrinogen contributions, with greater fibrinogen impairment after 60 min shock from widespread tissue hypoperfusion.

2. Materials and methods

2.1. Animals and reagents

Male Sprague-Dawley rats (300–450 g; Monash University, Australia) were housed in a 14–10 h light-dark cycle with free access

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to food/water. Thiobarb (Na-thiopentone) anesthesia was administered intraperitoneally (100 mg/kg) and as required throughout the protocol. The study conformed to the *Guide for Care and Use of Laboratory Animals* (NIH, 8th Edition, 2011) and was approved by JCU Ethics Committee (A1646). Thiobarb and 2% lidocaine-HCl were obtained from Lyppards (Townsville, QLD), and other chemicals from Sigma Aldrich (Castle Hill, NSW).

2.2. Surgical protocol

Following anesthesia, a tracheotomy was performed and animals ventilated at 90–100 strokes/min with PEEP of 1 cm on humidified room air (Harvard Small Animal Ventilator, Holliston, USA) [24,25]. Temperature was monitored with a rectal probe. The left femoral vein and artery were cannulated using PE-50 tubing for infusions and hemodynamics (Powerlab, ADInstruments, Australia) [21], and the right femoral artery was cannulated for blood withdrawal and sampling [26]. All perfusion lines were coated with citrate-phosphate-dextrose (CPD) on the luminal surface to ensure patency.

2.3. Experimental design

Non-heparinized rats were randomly assigned to one of three independent groups: 1) Baseline, 2) Bleed, or 3) Shock (Fig. 1A) ($n = 10$ each group). Animals were sacrificed for coagulation analysis: i) following surgery prior to bleed (Baseline), or ii) after 20 min controlled bleed (Bleed), or iii) after 60 min shock (Shock). An independent-groups study was required because of the limited volume of blood in the rat after hemorrhage and the volume of blood required for the assays.

2.4. Shock protocol

Hemorrhagic shock was induced by withdrawing blood from the femoral artery to reduce MAP to between 35 and 40 mm Hg, and as MAP compensated more blood was removed. This process was continued over 20 min. Shed blood was kept in a syringe at room temperature (22 °C) in the presence of 0.7 ml CPD/10 ml blood. Average shed volume was 12.3 ± 0.4 ml which equated to an average blood loss of 42 ± 0.7%.

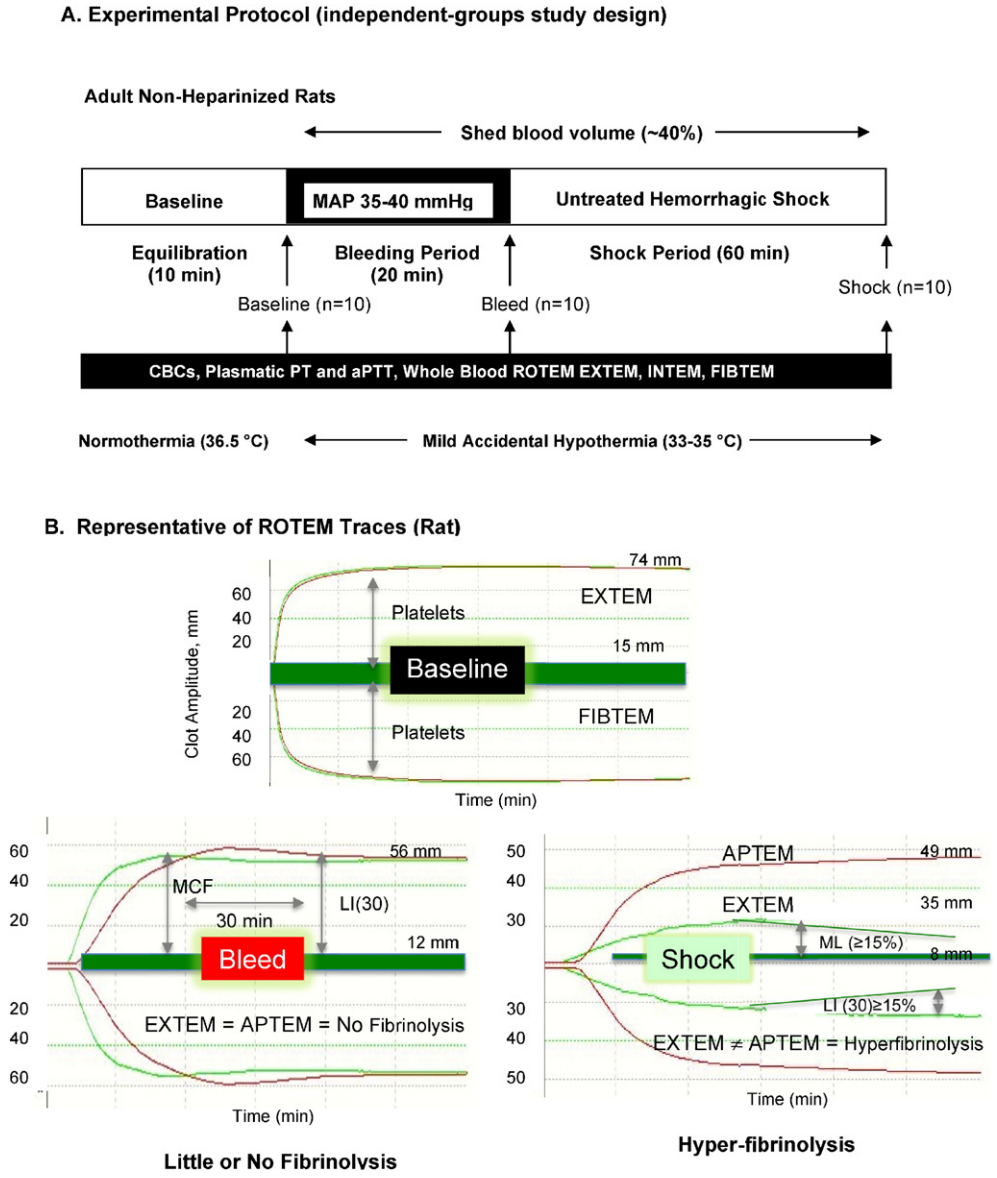

Fig. 1. A: Schematic of the in vivo rat model of pressure-controlled bleeding and severe hemorrhagic shock. Rats were not heparinized and body temperature was allowed to drift. B: Representative ROTEM temograms for EXTEM, FIBTEM and APTEM tests at baseline (Baseline), after 20 min bleeding (Bleed), and after 60 min untreated shock (Shock). ROTEM parameters include time to initiate clotting; time of clot formation; alpha angle; clot amplitude; maximum strength of clot; maximum clot elasticity, maximum clot velocity; and a number of lysis indices (see Materials and methods).