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Fresh frozen plasma: red blood cells (1:2) coagulation effect is equivalent to 1:1 and whole blood

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ABSTRACT

Background: Preemptive treatment of trauma-associated coagulopathy involves transfusion of fresh frozen plasma (FFP) at 1:1 ratio with red blood cells (RBCs), but the optimal ratio remains controversial. In combat theaters, fresh whole blood (FWB) is also an option. The objective of this study was to determine the effect of FFP:RBC ratios 1:1, 1:2, 1:3 and FWB on coagulation during resuscitation.

Materials and methods: Thirty-six rats were randomized in the following six groups: Group 1: sham; Group 2: hemorrhage followed by sole lactated Ringer (LR) infusion; Group 3: FFP:RBC (1:1); Group 4: FFP:RBC (1:2); Group 5: FFP:RBC (1:3); Group 6: FWB transfusion. Another 25 animals were used for blood harvesting. Hemorrhage was induced by withdrawing 40% of total blood volume, mean arterial pressure (MAP) decreased to 45% of baseline, and laparotomy. Animals underwent LR infusion followed by blood product transfusion preset for each group. Blood samples were obtained at baseline and in the 105th minute for thromboelastometry and lactate.

Results: Hemorrhage caused a significant decrease in MAP and increase in lactate ($P < 0.05$). MAP was persistently low in group 2 despite fluid infusion ($P < 0.05$), but not in the other groups after 20 min of resuscitation. Mean clot formation time, alpha angle, and maximum clot firmness decreased significantly ($P < 0.05$) in group 2 (LR) and group 5 (1:3) compared with other groups.

Conclusions: FFP:RBC in a 1:2 ratio optimally harnessed hemostatic resuscitation and prudent use of blood products compared with 1:1 and 1:3 ratios and to FWB transfusion.

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1. Introduction

Although hemorrhage is the most important cause of preventable death in trauma, it is still responsible for approximately 50% of the deaths that occur within the first 48 h after injury [1]. Severe hemorrhage leads to coagulopathy, which is present on admission in approximately 25% of severely injured patients. That condition is associated with a four-fold increase in the risk of death [2–4]. Preemptive treatment of trauma-associated coagulopathy (TAC) was shown to reduce mortality in hemorrhaging patients [4]. That strategy involves fresh frozen plasma (FFP) and platelets transfused at a near 1:1:1 ratio with red blood cells (RBCs) [4,5]. Furthermore, a high fibrinogen (>0.2 g) and/or cryoprecipitate:RBC ratio was also shown to improve survival in both military and civilian trauma settings [6,7]. More recently, the use of fresh whole blood (FWB) was revisited in combat theaters as full-scale treatment option of coagulopathy [8,9]. Studies have shown that the use of FWB is logistically more appealing in the military setting despite scarce data to compare that strategy with component therapy [8,9].

For the most part, several aspects relative to the management of TAC need further investigation, including acute and long-term complications associated with blood product transfusion [10–12]. However, inherent limitations of previous clinical trials create significant obstacles to determine what therapy most effectively addresses TAC with fewer complications [13–15]. Hence, animal models provide important means to answer part of those questions experimentally [16].

Our hypothesis was that in a controlled experimental setting, FFP and RBC in ratio of 1:1 and FWB transfusions would have similar effects on coagulation compared with FFP:RBC in a ratio of 1:2. Furthermore, we speculated that FFP:RBC in a ratio lower than 1:2 and sole infusion of crystalloids would interfere with coagulation in hemorrhage resuscitation.

The objective of the present study was to determine the effect of FFP:RBC in ratios of 1:1, 1:2, 1:3 and FWB transfusion on coagulation in an experimental model of hemorrhage resuscitation.

2. Materials and methods

The study was approved by the Animal Research Committee of the Federal University of Minas Gerais, Brazil under protocol number 193/2011. Male Wistar rats (200–300 g) were acclimated for 2 wk, individually housed, and maintained at 25°C on 12-h light/day cycles. Animals were fed rat chow (Labina; Purina, Caxias, Brazil), and water *ad libitum*.

2.1. Prehemorrhage procedures

Animals were anesthetized with ketamine 60 mg/kg and xylazine 15 mg/kg (Fort Dodge Animal Health, Fort Dodge, IA) administered by intraperitoneal injection. Additional intravenous doses were given if needed. A tracheostomy was performed; and subsequently, the left internal jugular vein and the right carotid artery were cannulated with 24-gauge peripheral intravenous catheters previously filled with saline (Smiths Medical, Sao Paulo, Brazil). Mean arterial pressure (MAP) was recorded continuously with a monitor (ProPaq Protocol Systems, Beaverton, OR) connected to the right carotid artery. All animals underwent a midline laparotomy (4 cm) to incorporate tissue injury to the model. The bowel was eviscerated for 60 s followed by abdominal closure with running 2-0 nylon suture.

A blood sample (1 mL) was obtained from the right carotid artery for baseline thromboelastometry (ROTEM Coagulation Analyzer; Pentapharm, Munich, Germany). The sample was immediately transferred to a tube containing anticoagulant (sodium citrate 3.2%— MiniCollect, Vacuette, Monroe, NC). Thromboelastometry was performed on temperature-corrected blood samples using nonactivated TEM (NATEM; Pentapharm, Munich, Germany) and star-tem (Pentapharm, Munich, Germany) reagent to recalcify citrated blood (Ref. Number 503-01). Thromboelastometry parameters were calculated with the coagulation dynamics evaluation software (DyCoDerivAn; Avordusol, Risskov, Denmark). The following parameters were assessed: clotting time (CT), clot formation time (CFT), alpha angle (α), maximum clot firmness (MCF), and maximum lysis (ML) at 60 min. Two separate blood samples (1 mL each) were obtained for assessment of lactate levels (Biosen C-Line; EKF Diagnostics, Cardiff, UK), one at baseline and the other 15 min after the beginning of hemorrhage.

2.2. Experimental groups

Thirty-six animals ($n = 36$) were randomly divided into six groups ($n = 6$ per group) according to the following resuscitation regimens:

- Group 1 (G1; sham): Animals underwent surgical procedures but no hemorrhage.
- Group 2 (G2): resuscitation with lactated Ringer (LR) infusion only.
- Group 3 (G3): resuscitation with LR and FFP:RBC (ratio 1:1).
- Group 4 (G4): resuscitation with LR and FFP:RBC (1:2).
- Group 5 (G5): resuscitation with LR and FFP:RBC (1:3).
- Group 6 (G6): resuscitation with LR and FWB.

All animals received LR during resuscitation to simulate current clinical practice in trauma care.

2.3. Procurement and preparation of RBC, FFP, and FWB for transfusion

Another 25 animals (200–250 g) were used specifically for blood product procurement. All blood products were transfused on the same day within 6 h of preparation. Animals were anesthetized as previously described. The right internal carotid artery was cannulated with a 24G peripheral intravenous catheter for blood withdrawal. The total blood volume (TBV) procured was calculated through the formula ($TBV = 0.06 \times \text{weight (g)} + 0.77$) and was immediately placed in tubes containing 3.2% sodium citrate; the tubes were centrifuged at 2000 rpm for 19 min. The supernatant (FFP) was