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Procoagulant activity in stored units of red blood cells

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ABSTRACT

The procoagulant activity (PA) of stored units of red blood cells (RBC) increases over time, which is related to the expression/exposure of tissue factor (TF). However, there is a discrepancy between the TF measured and changes in PA observed, suggesting that other blood components contribute to this activity. Our goal was to evaluate changes in PA of stored RBCs and to determine possible contributors to it. RBC units from 4 healthy donors were prepared and stored at 4 °C. On selected days, RBC aliquots were reconstituted with autologous plasma and tested in the thromboelastography assay. Corresponding supernatants were tested in a clotting assay. For all donors, the clotting time (CT) of reconstituted RBC units decreased from ~3000–4000s on day 1 to ~1000–1600s on day 30, with the most dramatic changes occurring between days 1 and 5. Anti-TF antibody slightly prolonged the CT. The concentration of TF did not change significantly over time and was within the range of 0.3–2.3 pM. Bovine lactadherin (LTD) prolonged the CT of the RBC (by 2.4–3.4-fold in days 3–5 and by 1.3–1.8-fold at day 30). Anti-TF antibody together with LTD had a cumulative effect on the CT prolongation. CT of supernatants responded to both anti-TF and anti-FXIa antibodies. Three contributors to the PA of stored RBC were identified, i.e. FXIa in solution and phosphatidylserine and TF exposed on blood cells and microparticles. Failure of LTD and antibodies to completely eliminate PA suggests that other components of blood could contribute to it.

1. Introduction

Commonly used transfusion products include crystalloid, packed red blood cells (RBC), plasma and platelets [1,2]. Although fresh frozen plasma is the most commonly used hemostatic agent worldwide [3], more recently a combination of all three blood products (RBC, plasma and platelets) at various ratios with a simultaneous decrease in crystalloid use has become more popular [4,5]. Some data suggest that high transfusion ratios of fresh frozen plasma and platelets are beneficial for survival in patients with coagulopathy [6], although there is no consensus on this subject and further studies are required [7]. In addition to blood products, several adjunct agents have been evaluated for the bleeding control in trauma induced coagulopathy, such as fibrinogen concentrates, prothrombin complex concentrates, recombinant factor VIIa and antifibrinolytic agents [7–11]. Some of the adjunct products showed promising results [11,12], whereas the use of others either did not provide conclusive results [10], or have been potentially associated with thrombotic risk [9,13].

Approximately 14 million units of whole blood and RBC are transfused in the United States annually [14]. Freshly prepared units of RBC are refrigerated and stored for up to 42 days at 1–6 °C. However, stored RBC undergo functional and structural changes/deterioration, which collectively are called storage lesion [15]. It has been suggested in several studies that transfusion of old RBC associates with an increased morbidity and mortality [16,17], although this subject remains controversial [18]. However it is almost commonly accepted that RBC storage increases their procoagulant activity, which is primarily assigned to the exposure of an acidic phospholipid, phosphatidylserine, on the surface of RBC [19,20] or present on microparticles shed by blood cells [21–23]. In addition, tissue factor (TF) located on microparticles and blood cells could provide an additive input to the procoagulant activity of stored RBC [23].

In the current study, the effect of RBC storage on changes in their procoagulant activity over time was evaluated and several causes of that activity were established.

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2. Materials and methods

2.1. Materials

Bovine lactadherin (LTD) and human factor (F)XIa were purchased from Haematologic Technologies, Inc. (Essex Junction, VT). Trypsin inhibitor from corn (CTI; prevents contact pathway initiation of coagulation) was prepared as previously described [24]. Recombinant human TF_{1-243} was a gift from Dr. R. Lundblad (Baxter Healthcare Corp., Duarte, CA). It was relipidated into phospholipid vesicles (PCPS) composed of 25% dioleoyl-*sn*-glycero-3-phospho-L-serine and 75% of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (both from Avanti Polar Lipids, Inc; Alabaster, AL) prepared as described previously [25]. Inhibitory monoclonal anti-TF (αTF-5; prevents binding of TF to FVIIa) and anti-FXIa (αFXI-2; inhibits FIX activation by FXIa) antibodies were produced and characterized in-house [26,27].

2.2. Preparation of RBC units

In accordance with a protocol approved by the institutional review committee at the University of Vermont, blood was taken from 4 healthy volunteers by phlebotomy from an antecubital vein after the volunteer gave written informed consent. 450 ml blood were collected following standard Blood Bank procedures for generating autologous units of RBC. RBC units were prepared by centrifugation at 5000× g for 5 min. The 450 ml collection bag (Haemonetics, Braintree, MA) contained 63 ml of CPD solution (1.61 g dextrose, 1.66 g sodium citrate, 188 mg citric acid and 140 mg monobasic sodium phosphate; all from Fisher Scientific, Fair Lawn, NJ). Autologous plasma was removed, aliquoted and stored at −80C for the duration of the experiment. After the plasma removal, 100 ml of Optisol, (877 mg sodium chloride, 900 mg dextrose, 525 mg mannitol and 30 mg adenine) was added. After the first sample was removed for analysis, RBCs were stored at 4 °C. The first 5 ml sample (Day 1) was removed by a syringe after RBC preparation (before refrigeration) and divided into two 2.5 ml aliquots. One aliquot was reconstituted with 2.5 ml autologous plasma and analyzed by thromboelastograpy. The second aliquot was used for the supernatant preparation. For subsequent time points (days 1–32), a 5 ml aliquot was taken from the refrigerated RBCs, warmed for 15 min at 37 °C and divided into two 2.5 ml aliquots. One aliquot was reconstituted with 2.5 ml warmed autologous plasma and analyzed by thromboelastograpy and the second aliquot was used for the supernatant preparation.

2.3. Thromboelastography

Procoagulant activity of reconstituted RBC was tested in the thromboelastography assay using 4-channel Delta Analyzers from TEM systems (Durham, NC). CTI was added at a final 0.1 mg/ml concentration to reconstituted RBC aliquots and aliquots were placed into thromboelastograph's cups and recalcified with 1 M $CaCl_2$ to a final 19.5 mM concentration. In parallel, αTF-5 at a final 0.1 mg/ml concentration or bovine lactadherin at a final 100 nM concentration were added to other aliquots of reconstituted RBC prior to the addition of $CaCl_2$. Thromboelastographic measurements were done in duplicates and recorded for 90 min. The concentration of active endogenous TF was quantitated from the prolongation of the clotting time caused by α-TF-5. Similarly, procoagulant activity related to the phosphatidylserine exposure was determined from the prolongation of the clotting time caused by the addition of bovine lactadherin. In TF control experiments, relipidated TF was added at final 0.5 and 5.0 pM concentrations.

2.4. RBC supernatant clotting assay

Analysis of procoagulant activity in the supernatant from stored RBC was performed in a clotting assay. At selected days (1–32), stored RBC preparations were centrifuged at 800× g for 15 min at room temperature. The supernatant was removed, CTI was added at a final 0.1 mg/ml concentration and supernatant was divided into two 1.25 ml aliquots. One aliquot was analyzed immediately for the TF and FXIa activity and the second aliquot was centrifuged at 1,00,000× g for 1 h. TF and FXIa activity in supernatants were measured as previously described [28]. Briefly, $CaCl_2$ was added to supernatants at a final 12 mM concentration and incubated for 1 min at 37 °C; clotting was initiated by 2 μM PCPS. In parallel, αTF-5 or αFXI-2 at a final 0.1 mg/ml concentration was added to an aliquot of supernatant prior to the addition of $CaCl_2$. Clotting times were determined using a clotting instrument ST-8 from Diagnostica Stago (Parsippany, NJ). Supernatant samples that had not clotted in 2000 s (the upper limit of the assay) in the absence of αTF-5 and αFXI-2 were considered as not having procoagulant activity. TF and FXIa activity in supernatant were calculated from the prolongation in clotting times in the presence of αTF-5 or αFXI-2, respectively, using calibration curves developed with relipidated TF_{1-243} or FXIa in pooled 10-donor normal plasma.

3. Results

3.1. Changes in procoagulant activity of reconstituted RBC over time

The clot time (R-time) varied for 4 preparations of RBC in the thromboelastography assay in a wide range (Fig. 1). The shortest clot time (2089 s) observed at day 1 in the presence of CTI was for the donor 2 and the longest clot time was measured for the donor 4 (4049 s), with reconstituted RBC from donors 1 and 3 showing clot times of 3582 and 3105 s, respectively. The clot time for the RBC from donor 2 was the shortest through the entire 30 days of storage and changes in the clot times for all donors occurred at varying rates. The longest clot time at day 30 was observed for the RBC from donor 4 (1615 s). Overall, the drop in the clot times for all 4 donors over 30 day period (32 days for donor 1) was quite significant (up to 3.7-fold), with the most dramatic changes occurring within the first 5–10 days.

A response of RBC clot time to the TF-challenge and changes in

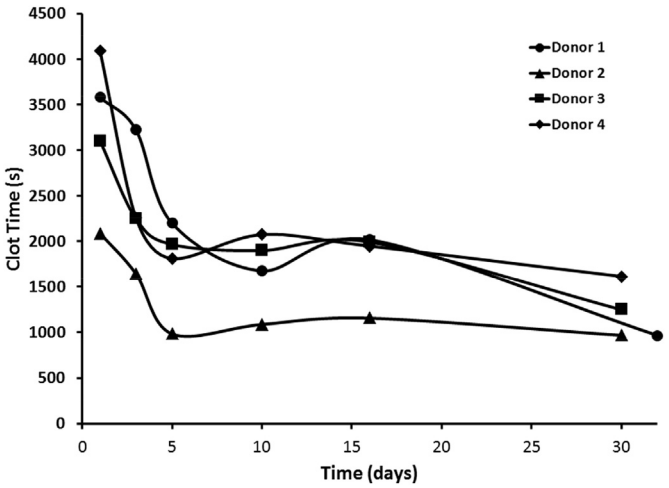

Fig. 1. Clotting time of stored RBC units over storage time in the presence of 0.1 mg/ml CTI. Curves represent donor 1(●), donor 2 (▲), donor 3 (■) and donor 4 (◆).

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