Systemic and microvascular effects of resuscitation with blood products after severe hemorrhage in rats

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BACKGROUND: Severe hemorrhage is associated with the disruption of the endothelial glycocalyx (EG), a key component of the endothelium. The effects of blood components on the EG are unknown. The present study furthers our investigations into the effects of resuscitation with blood products on the skeletal muscle microcirculation of hemorrhaged rats, focusing on packed red blood cells (PRBCs) or fresh whole blood (FWB).

METHODS: Rats were bled 40% of total blood volume and resuscitated with 1:1 PRBC/lactated Ringer's solution (LR), 1:1 washed PRBC (wPRBC)/LR, FWB or LR only. Sham animals were subjected to all procedures except hemorrhage and resuscitation. EG thickness, blood flow, and microvascular permeability were studied using intravital microscopy. Hemodynamics and coagulation tests (rotational thromboelastometry) were performed.

RESULTS: After severe hemorrhage, EG and permeability were restored to sham levels in the PRBC/LR and FWB groups, but not in the wPRBC/LR or LR groups. Clotting time was longer and clot elasticity and firmness were reduced in wPRBC/LR and LR, but not in FWB or PRBC/LR groups when compared with sham.

CONCLUSION: Resuscitation with FWB or PRBC/LR was superior in reversing coagulopathy, restoring EG and permeability changes following hemorrhage, compared with wPRBC/LR or LR alone. As wPRBC/LR did not improve EG and permeability, these data suggest that the removal of residual plasma protein from wPRBC or resuscitation with a protein-free solution (LR) is not able to improve microcirculation and coagulation functions in this severe hemorrhage model. (*J Trauma Acute Care Surg*. 2014;77: 716–723. Copyright © 2014 by Lippincott Williams & Wilkins)

KEY WORDS: Microcirculation; packed red blood cells; intravital microscopy; ROTEM; plasma proteins; rats.

Current US military guidance for the resuscitation of war fighters with life-threatening injuries in deployed US surgical facilities is to achieve early hemorrhage control and resuscitate with blood components in equal proportions to whole blood (1:1:1 U ratio of packed red blood cell [PRBC]/plasma/platelets) while minimizing the use of crystalloids or synthetic colloids.[1] However, component use in austere combat settings is often limited by storage requirements for blood products.[2,3] Therefore, military clinical practice guidelines support the use of fresh whole blood (FWB) in casualties anticipated to require a massive transfusion when component therapy is unavailable or fails to reverse coagulopathy in life-threatening scenarios.[3–5] Retrospective studies suggest that transfusion outcomes in patients who received FWB were equivalent or better compared with those treated with component therapy, and logistic regression identified FWB use as an independent marker associated with survival.[6]

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While the mechanisms underlying this finding have not been elucidated, recent reports of effects on endothelial function may provide one clue. At the microvascular level, hemorrhagic shock is associated with disruption of endothelial cells, apoptosis, and increased microvascular permeability.[7] The endothelial glycocalyx is a key component of the endothelial cell since it serves as a barrier to transvascular exchange of water and solutes[8] and filters plasma-borne macromolecules.[9] Glycocalyx thickness is a reliable and useful estimate of the structural organization and stability of individual glycocalyx components, such as glycoproteins, proteoglycans with glycosaminoglycans, and adsorbed plasma proteins.[10–12] Previously, we showed that an in vivo model of hemorrhagic shock results in shedding of skeletal muscle and mesentery venule glycocalyces.[13] Reduction in glycocalyx thickness may be associated with loss of the endothelial barrier, which may in turn leads to secondary tissue injury and dysfunction, including edema, inflammation, and coagulation abnormalities. In vivo reconstitution of the endothelial glycocalyx is important to preserve endothelial cell viability and function by (1) maintaining a protective cushion of glycocalyx macromolecules to keep the circulating RBCs away from endothelial cell surface, (2) preventing the adhesion of neutrophils and platelets to the endothelium, (3) acting as a mechanosensor on the endothelial cell, and (4) affecting the transvascular exchange of fluids and solutes.[12,14–17] In addition, increased levels of syndecan 1, a marker for glycocalyx disruption, may predict mortality in trauma patients.[18] We previously demonstrated in vivo that complete reconstitution of the glycocalyx after hemorrhagic

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shock can be achieved by small-volume resuscitation (15 mL/kg) with fresh frozen plasma (FFP), but not with colloids.[19] Moreover, Kozar et al.[20] reported that shock-induced glycocalyx degradation observed via electron microscopy was partially repaired in rodents treated with resuscitation using plasma, but not lactated Ringer's solution (LR). The effects of other blood products on glycocalyx restoration after shock have not been documented. Here, we continue our effort to characterize blood products used by the military with regard to the impact on the microcirculation and the endothelium. We investigated the effects of small-volume PRBC/LR resuscitation versus washed PRBC (wPRBC)/LR, FWB, and LR alone on glycocalyx thickness, blood flow, microvascular permeability, glycocalyx components in plasma, microhemodynamics, as well as coagulation and systemic parameters. Our hypothesis was that resuscitation with PRBC would not restore microvascular integrity or maintain coagulation function compared with FWB.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research and conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

Systemic Measurements

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA; body weight, 220 ± 7 g) breathing 100% O_2 were anesthetized with isoflurane (2%) and tracheostomized. The carotid artery was cannulated (SPR-320, Millar Instruments, Houston, TX) for recording blood pressure. A femoral vein and artery were cannulated (PE 50, Instech Laboratories, Inc., Plymouth Meeting, PA) for fluorescent dye infusion and blood withdrawal, respectively. Heparinized arterial blood was used to measure hematocrit, pH, lactate, base excess, bicarbonate, and K+ (CG4, CHEM8 modules, I-stat, Abbott, Chicago, IL), and heparan sulfate levels. Hemoglobin O_2 saturation (SO_2) and respiratory rate (RR) were measured noninvasively (MouseOx, Starr Lifesciences, Pittsburgh, PA). Mean arterial blood pressure (MAP), heart rate, core temperature, SO_2, and RR were recorded continuously (Dynamic Research Evaluation Workstation, USAISR, San Antonio, TX).[13] The first milliliter of hemorrhaged blood was used for coagulation assays (rotational thromboelastometry [ROTEM], TEM Innovations GmbH, Germany).

Intravital Microscopy and Animal Preparation

A microscope (BX51WI, Olympus) with an immersion objective (Zeiss 63x, N.A. 0.95) and filter sets for Texas Red (TR) and fluorescein isothiocyanate (FITC) fluorescence was connected to a velocity-measuring online device (Optical Doppler Velocimeter, College Station, TX) and to a digital camera (CoolSnap CF, Roper Scientific, NJ).[13] The cremaster muscle was exposed over a heated pedestal and covered with an impermeable plastic film.[21] The animal platform was then positioned over the microscope stage.[13]

Washing PRBC Units

After whole blood was collected from each donor rat and centrifuged at 5,000 G for 5 minutes, half of the PRBCs underwent a washing protocol and the other half remained unwashed. Unwashed PRBCs were resuspended in AS-5 (Baxter Healthcare Corp., Deerfield, IL) at a 1.8:1 PRBC/AS-5 ratio and kept at 4°C. wPRBC were diluted 1:1 (vol./vol.) with AS-5 and centrifuged at 5,000 rpm for 1 minute at 4°C. This was repeated three times. Lastly, the supernatant was discarded and AS-5 was added to the PRBC pellet at a 1.8:1 ratio and kept at 4°C. To standardize results between study arms, all PRBC units were used within 2 days of blood collection, with one exception. That unit was used 3 days after collection.

Fresh Whole Blood

Citrated FWB was collected from donor rats, stored at 4°C, and used within 24 hours of collection.

Fluorescently-Labeled Dextran (Dx) Solutions

Dextrans labeled with TR or FITC (Dx70, 70 kD; Dx500, 500 kD) were used to estimate the glycocalyx thickness.[13] At baseline, TR-Dx70 (10 mg/mL, Molecular Probes, Life Technologies, Carlsbad, CA) was injected to document glycocalyx thickness, followed by FITC-Dx500 (10 mg/mL, Sigma-Aldrich, St. Louis, MO) after HS/resuscitation.

Permeability Index Measurements

Microvascular permeability to FITC-labeled bovine serum albumin (FITC-BSA, 20 mg/mL, Sigma-Aldrich) was determined as described previously.[22] Briefly, after FITC-BSA injection, images of selected vessels were captured using an image-processing software (ImagePro Plus 7, Media Cybernetics) to assess the presence of visible fluorescent leaks. The leakage was quantified offline (Image J software, NIH, Bethesda, MD) by measuring the fluorescence intensity of six areas within the venules (Iv) and in the perivenular interstitium (Ip). The permeability index (PI) was calculated as the ratio between Ip and Iv (Fig. 1).

Experimental Protocol

Approximately four to six microscopic fields containing postcapillary venules were selected for analysis. Brightfield and fluorescence images of each field were captured using the CCD camera and transferred to a computer hard drive 5 minutes after the TR-Dx70 injection. RBC velocity was determined online. Blood flow and wall shear rate were calculated from RBC velocity and vessel diameter.[13] A fixed-volume hemorrhage was performed during a 30-minute period (T0 − T30) to a target of 40% of total blood volume.[13] After an additional 30 minutes (T30 − T60) of hemorrhagic hypotension, animals were randomized into four resuscitation groups and a sham: (1) resuscitation with FWB (15 mL/kg); (2) 1:1 PRBC/LR (15 mL/kg); (3) 1:1 wPRBC/LR (15 mL/kg); and (4) LR, (75 mL/kg). Military units in dispersed areas may carry up to 4 U of blood products on helicopters, including FFP and RBCs, to administer as an initial resuscitation fluid in casualties far forward.[23] The dose of 15 mL/kg is equivalent to 4 U of blood products and ensures concomitant volume expansion and hypotensive resuscitation. One hour after resuscitation (T60 − T120), FITC-Dx500 was injected, and paired recordings of the selected fields were performed. Two sets of systemic parameters were collected coinciding with the microcirculatory data at baseline and after resuscitation. Two samples of arterial blood

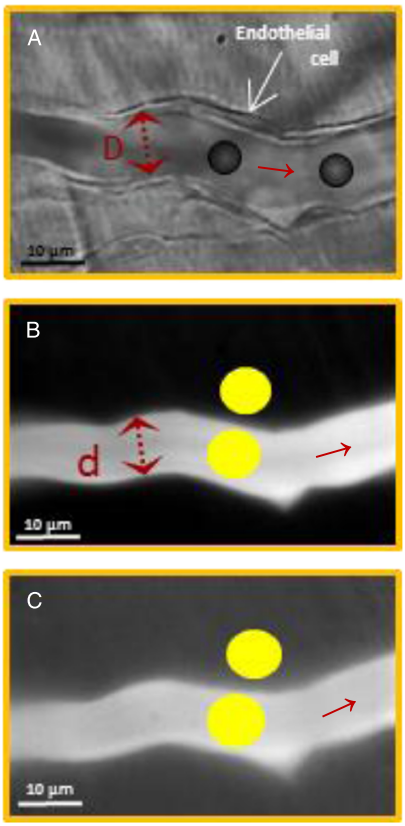

Figure 1. *A,* Transillumination in vivo image of a postcapillary venule in a rat cremaster. The *white arrow* points to the endothelial cell on the vessel wall. *D* represents the anatomic (luminal) diameter of the venule. The *black dots* denote the photo diodes of the optical velocity device to measure RBC velocity online. *B,* Epi-illumination in vivo image after injection of TR-Dx70. *d* represents the diameter of the fluorescent column. Glycocalyx thickness was calculated as the difference between *D* and *d*. The *yellow dots* denote intravascular and perivascular areas where the light intensities are measured. *C,* Epi-illumination in vivo image after injection of FITC-BSA for quantification of microvascular permeability. PI was calculated as the ratio of perivascular and intravascular light intensities.

were withdrawn after microcirculatory measurements were complete at baseline and after resuscitation (T120). Control animals (sham) were subjected to all procedures except hemorrhage and resuscitation.

Plasma Levels of Heparan Sulfate

Previously, frozen plasma samples were thawed and used to analyze rat heparan sulfate using a commercial enzyme-linked immunosorbent assay kit (ABIN416454, Antibodies Online, Atlanta, GA).

Thromboelastometry

A functional assay of blood clotting was performed using ROTEM. The extrinsic pathway (EXTEM) was studied in citrated blood samples, and platelet and fibrinogen contributions were discriminated with the FIBTEM assay. Parameters measured were clotting time (s), maximum clot firmness (mm), and maximum clot elasticity (dynes/cm^2).

Glycocalyx Thickness Measurements

The glycocalyx thickness was measured as previously described by our group.[13] Briefly, epi-illumination and transillumination images were used to measure the fluorescent column width and the microvessel anatomic (lumen) diameter. The thickness was estimated by the difference between these two measurements (as illustrated in Fig. 1). Image processing was performed using Image-Pro Plus software (MediaCybernetics, Rockville, MD).

Statistical Analysis

We used SigmaPlot 12 (2011 Systat Software, Inc., San Jose, CA) for the statistical analysis. Deviation from Gaussian distribution was tested for systemic and microvascular data using the Shapiro-Wilk test. All data were amenable to parametric testing. Values were reported as mean ± SEM. Differences between samples obtained before and after severe hemorrhage and among groups were analyzed using repeated-measures analysis of variance followed by multiple comparison procedures (Student-Newman-Keuls). The values were considered to differ significantly if $p < 0.05$.

RESULTS

Systemic Responses

All rats were bled an average of 25 ± 0.8 mL/kg. Table 1 shows the systemic parameters (n = 25) measured at baseline, end of hemorrhage (T30), and after 30 minutes of hypotension (T60). The severity of the hemorrhage was evidenced by the rise in plasma lactate and potassium and the fall in bicarbonate and base excess at T30 and T60. However, all variables returned to prehemorrhage levels after resuscitation treatments (data not shown), except MAP. Because of the nature of our resuscitation protocol (hypotensive), the postresuscitation MAP was significantly lower in the PRBC/LR, wPRBC/LR, LR and FWB groups (78 ± 5.8, 80 ± 4.3, 82 ± 3.2 and 80 ± 3.7 mm Hg, respectively) compared with sham (102 ± 8 mm Hg).

The mean baseline hematocrit (n = 25) was 42% ± 1%. Hematocrit levels were higher in rats that received 15 mL/kg of FWB or PRBC (Fig. 2*A*) compared with the LR group ($p < 0.05$). Those in sham animals were unchanged at the "postresuscitation" time point, averaging 39% ± 2%. Figure 2*B* shows that the total plasma protein of rats treated with LR or wPRBC/LR (3.1 ± 0.1 g/dL and 3.6 ± 0.1 g/dL, respectively) was lower compared with that of animals treated with FWB or PRBC (3.9 ± 0.1 g/dL and 3.8 ± 0.2 g/dL, respectively) as well as sham rats ($p < 0.05$). Total protein and albumin levels measured in the FWB were 5.6 g/dL and 1.0 g/dL, respectively. In the unwashed PRBC, the levels of total protein and albumin were respectively 2.7 g/dL and 0.5 g/dL, whereas the wPRBCs had only 1.8-g/dL total protein and undetectable levels of albumin.

Severe hemorrhage followed by resuscitation with wPRBC/LR and LR led to the prolongation of the clotting time (Fig. 3*A*) and lower maximum clot elasticity compared with those of sham and FWB groups (Fig. 3*C*). Maximum clot elasticity measured in sham and FWB-treated rats remained

TABLE 1. Systemic Parameters Before and After Severe Hemorrhage

Parameter	Baseline	Hemorrhage (T30)	Hypotension (T60)
MAP, mm Hg	100.5 ± 1.7	46.0 ± 2.8*	55.0 ± 3.1*
Pulse pressure, mm Hg	28.5 ± 3.1	47.7 ± 3.2	34.7 ± 5.9
Heart rate, beats/min	348 ± 11	355 ± 8	365 ± 12
O_2 saturation, %	99.4 ± 0.2	99.8 ± 1.7	99.1 ± 1.2
RR, breaths/min	66 ± 4	67 ± 3	70.2 ± 5
pH	7.368 ± 0.009	7.246 ± 0.006*	7.304 ± 0.013*
PO_2, mm Hg	378 ± 20	430 ± 10	417 ± 19
Base excess, mmol/L	1.8 ± 3.4	−4.1 ± 2.1*	−5.0 ± 7.0*
Bicarbonate, mmol/L	27.8 ± 1.3	24.3 ± 1.8	23.6 ± 1.8
Lactate, mmol/L	1.4 ± 0.5	5.1 ± 2.9*	5.4 ± 0.6*
Potassium, mmol/L	4.2 ± 0.2	5.3 ± 0.3*	6.1 ± 0.3*

*Significantly different from baseline ($p < 0.05$
Data are expressed as mean ± SEM for 25 animals.

unchanged in both EXTEM and FIBTEM assays after resuscitation (Fig. 3*B* and *C*).

Microvascular Responses

Ninety-eight venules were studied in five groups (n = 5 rats per group, 4–5 venules per rat). Anatomic diameter did not change among the groups, averaging 14.5 ± 3.4 μm and 13.9 ± 3.5 μm before and after severe hemorrhage/resuscitation, respectively. Baseline blood flow was not different among the groups (0.96 ± 0.14, 1.05 ± 0.20, 1.18 ± 0.20, 1.14 ± 0.22 and 1.02 ± 0.18 nL/s for sham, PRBC/LR, wPRBC/LR, LR only and FWB groups, respectively). After resuscitation, blood flow fell in FWB, PRBC/LR, and wPRBC/LR groups to 60%, 68%, and 56% of baseline, respectively, compared with sham (0.84 ± 0.11 nL/s, $p < 0.05$) but recovered in the LR group (1.07 ± 0.29 nL/s). The mean wall shear rate was 3.751 ± 0.432, 3.822 ± 0.684, 3.402 ± 0.553, 3.280 ± 0.462, 3.144 ± 0.708 × 10^3/s at baseline for sham, PRBC/LR, wPRBC/LR, LR only, and FWB groups, respectively. After severe hemorrhage/resuscitation, wall shear rate did not change in LR-treated rats (3.282 ± 0.735 × 10^3/s) but dropped in the FWB, PRBC/LR, and wPRBC/LR groups (65%, 75% and 49% of baseline, respectively) compared with sham (3.177 ± 0.432 × 10^3/s).

Endothelial Glycocalyx Thickness and Microvascular Permeability

The mean glycocalyx thickness (n = 25) at baseline was estimated as 0.571 ± 0.223 μm. Figure 4*A* shows that glycocalyx thickness was significantly lower in LR- and wPRBC/LR-treated rats (0.221 ± 0.032 μm and 0.231 ± 0.036 μm vs. 0.480 ± 0.052 μm, respectively) compared with sham and FWB after resuscitation (0.959 ± 0.104 μm and 1.084 ± 0.094 μm, respectively). We previously showed that glycocalyx thickness decreases with shock but postresuscitation values returned to near-baseline in PRBC/LR- and FWB-treated rats (0.562 ± 0.039 μm and 0.524 ± 0.065 μm, respectively).

Plasma levels of heparan sulfate, a marker of glycocalyx loss, in the FWB group were similar to sham and PRBC/LR levels after resuscitation (Fig. 4*B*). After hemorrhage/resuscitation with LR, heparan sulfate was significantly elevated compared with sham ($p < 0.001$). Higher levels of heparan sulfate after resuscitation with wPRBC/LR approached statistical significance (21.2% ± 1.0% higher than baseline, $p = 0.06$).

Figure 5 shows that resuscitation with LR increased microvascular permeability by more than 50%. Similarly, resuscitation with wPRBC/LR resulted in a small but significant increase in the PI (25.9% ± 0.1% from baseline). The PI returned to baseline levels when severe hemorrhage was treated with either FWB or unwashed PRBC.

DISCUSSION

This study furthers our investigation evaluating specific blood products used in damage-control resuscitation. We assessed the effects of PRBCs and FWB on microvascular function and coagulation in a murine model of hemorrhagic hypotension.[13,19] Resuscitation with FWB and PRBCs, but not wPRBCs, promoted microvascular stabilization by improving glycocalyx thickness, permeability barrier, and blood flow as well as improving hemostatic and hemodynamic parameters. Our data suggest that PRBC protective effects on the endothelium may be associated, at least in part, with the presence of residual plasma in RBC concentrates. Given that plasma induces glycocalyx structural recovery and prevents mobilization of components such as heparan sulfate or syndecan 1,[19] we hypothesize that residual plasma in PRBC is responsible for the favorable effects on glycocalyx thickness and permeability. These findings are supported by total plasma protein levels and glycocalyx thickness, both of which were significantly higher after resuscitation in the PRBC group compared with wPRBC. In addition, the coagulation status as measured by ROTEM was significantly better in the FWB and PRBC/LR groups, but not in the wPRBC/LR and LR groups. Since resuscitation using PRBC/LR and FWB showed similar endothelial glycocalyx recovery after severe hemorrhage and the latter contains platelets in addition to plasma, one can speculate that platelets may not be crucial to restoring glycocalyx thickness and PI to baseline levels. Therefore, this theory requires further investigation.

At postresuscitation period in all hemorrhaged groups, MAP was lower than baseline levels and compared with the sham group, as expected during hypotensive resuscitation.[24]

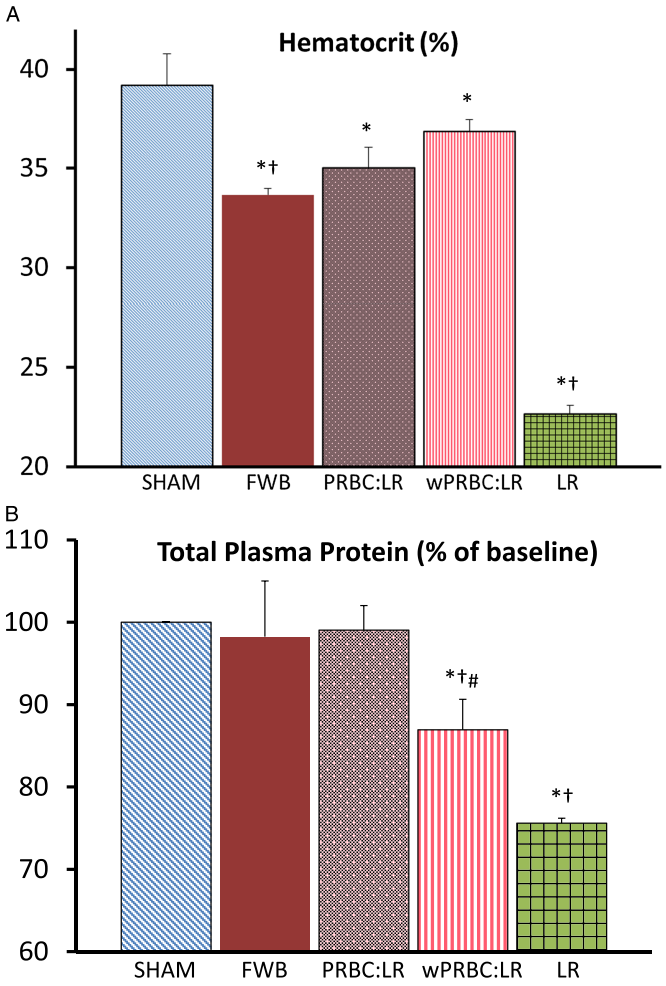

Figure 2. Hematocrit (*A*) levels after resuscitation and relative change in the levels of total plasma protein (*B*) for all the groups. *A*, Hematocrit levels in the FWB group were lower compared with baseline and with the sham group. Resuscitation with PRBC increased hematocrit significantly after hemorrhagic hypotension compared with LR. *B*, The presence of residual plasma in the PRBC group led to higher total plasma protein levels (4.0 ± 0.1 g/dL), compared with those in the wPRBC group (3.4 ± 0.2 g/dL). In the sham column, error bar is not visible because of their small size. Data are expressed as mean ± SEM. *Significantly different from baseline. †Significantly different from sham group. #Significantly different from PRBC/LR group.

Despite hypotension, all resuscitation treatments were able to correct metabolic acidosis caused by severe hemorrhage. We previously reported acid-base changes due to hemorrhage with or without resuscitation fluids in a model of hemorrhagic shock with anaesthetized rats.[13,19] In the current study, significant recovery of the acid-base balance to prehemorrhage levels was observed in all groups after resuscitation.

A fall in hematocrit has been described previously in rats after severe hemorrhage.[13,19] Hematocrit dropped by 77% from baseline in response to severe hemodilution caused by resuscitation with LR. In addition, hemodilution may account for the 24% fall in total plasma protein levels in LR-treated rats compared with baseline. The higher levels of total plasma protein in the PRBC group compared with wPRBC could be explained by residual plasma proteins in the unwashed PRBC. Damage to the RBC membrane or increased microparticles due to the washing procedure were not studied and cannot be ruled out as potential causes; however, with one exception, PRBCs were used within 2 days of collection to minimize the impact of the RBC storage lesion.

The irreversible RBC deterioration caused by the storage lesion is characterized by elevated potassium levels, hemolysis, bacterial overgrowth, and RBC-derived microparticles during storage and may be associated with reduced posttransfusion RBC survival/efficacy as well as increased risk of adverse

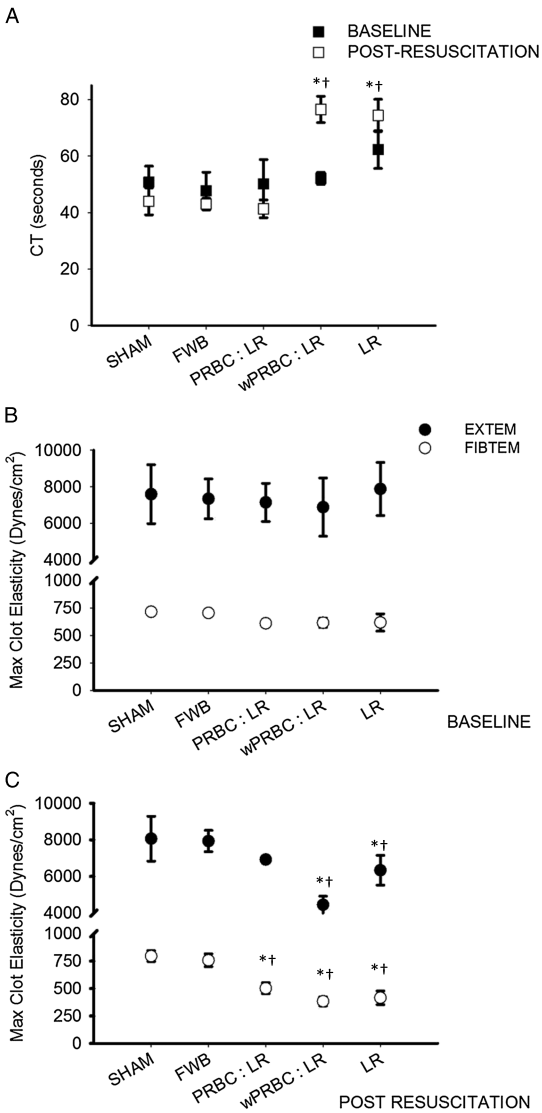

Figure 3. Relative change in clotting elasticity in the EXTEM assay (*A*) and FIBTEM (*C*) after hemorrhagic hypotension. *A*, LR and wPRBC decreased the elasticity of the clot compared with sham and FWB groups ($p < 0.05$). *C*, The clot elasticity was markedly lower in rats resuscitated with LR ($p < 0.05$) suggestive of dilution of the coagulation factors. Data are expressed as mean ± SEM. †Significantly different from sham group.

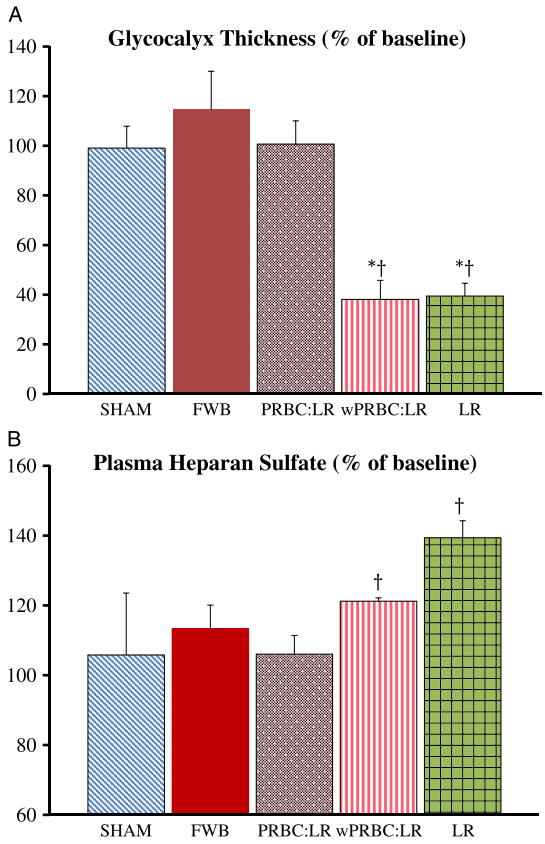

Figure 4. *A,* Endothelial glycocalyx thickness in postcapillary venules from cremaster preparations. *B,* Levels of plasma heparan sulfate proteoglycan relative to baseline after hemorrhage and resuscitation treatment. LR-treated rats had significantly higher levels of plasma heparan sulfate compared with sham (p <0.001). Data are expressed as mean ± SEM. *Significantly different from baseline. †Significantly different from sham group.

reactions in the recipients.[25,26] Although the age-related storage lesion was minimized in our protocol, the washing protocol used in the wPRBC group may have contributed to increased membrane instability and microparticle formation, thereby affecting the safety and effectiveness of the product.[27–29]

Thromboelastography or elastometry (ROTEM) is increasingly used to provide point-of-care results in both military and civilian trauma centers.[30,31] In this study, thromboelastometry demonstrated that clot strength decreases as evidenced by a fall in maximum clot elasticity. The groups treated with wPRBC and LR developed hypocoagulable characteristics, including prolonged clotting time. In the LR group, dilutional coagulopathy resulting in disordered clot polymerization has been described[32] and very likely played a role here, given the finding of very low plasma proteins. Interestingly, the volume of LR added to wPRBC, at 7.5 mL/kg, was approximately a tenth of that administered to the LR group and likely insufficient to be a major cause of postresuscitative coagulopathy. Despite the smallest change in hematocrit of the experimental (nonsham) groups, the plasma protein decrease in the wPRBC/LR animals was significant and exceeded only by the LR group. This finding indicates that autoresuscitation, which is described in the setting of unresuscitated hemorrhage and tissue injury,[33] may have been more of a factor in the group wPRBC/LR compared with either PRBC/LR or FWB .[34] The differences in plasma protein and glycocalyx integrity seen between the PRBC/LR and wPRBC/LR groups, both of whom received equal amounts of LR and overall volume, suggest that alterations in parameters of thromboelastometry in the latter group may be a function of mobilization of fluids into the vascular space, possibly owing to inflammatory changes associated with loss of endothelial integrity. The changes in coagulation observed in the wPRBC/LR and LR groups may thus be either attributable to or exacerbated by the attenuation of the protective glycocalyx layer and diffuse endotheliopathy, resulting in both mild consumption of platelets and coagulation factors, and in autodilution, which further compromises hemostasis. The higher levels of total protein and albumin in FWB and unwashed PRBC may provide the necessary substrate to allow glycocalyx restoration, precluding the progression of adverse effects of endotheliopathy on both coagulation and vascular function; to prevent and/or lessen secondary tissue injury; and to improve better clinical outcome after hemorrhagic shock.

Plasma-free resuscitation fluids (crystalloids) may lead to loss of glycocalyx-adsorbed proteins and proteoglycans and therefore increase microvascular flow,[32] which supports our findings that resuscitation with LR may cause shedding of glycocalyx followed by a significant increase in velocity (data not shown), blood flow, and wall shear rate in the vasculature after treatment with LR. Theoretical predictions on the mechanical properties of the endothelial glycocalyx suggested that the glycocalyx may cause an increase in blood flow resistance at low velocities in the microcirculation,[33] which may also explain the lower flow and shear rate after resuscitation with FWB as well as washed and unwashed PRBCs compared with sham. However, the RBC membrane lesion caused by the washing protocol may decrease RBC deformability and increase significantly the RBC transit time in the capillaries.[33,34]

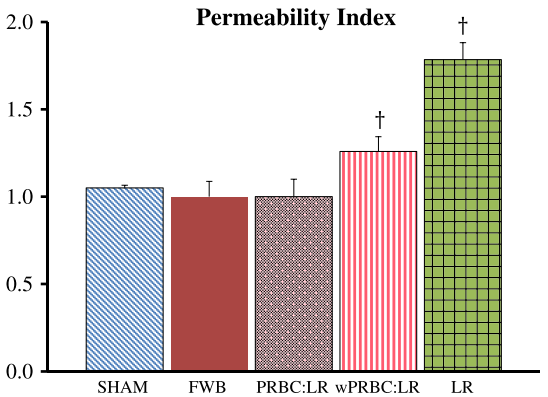

Figure 5. PI in the postcapillary venules (approximately 14 μm) from rat cremaster muscle. PI was calculated as the ratio of perivascular and intravascular light intensities. Highest microvascular permeability was found in the LR-treated rats. Data are expressed as mean ± SEM. †Significantly different from sham group.

Heparan sulfate is a prominent component of the glycocalyx that can bind to protein backbones (syndecans and glypicans) and form proteoglycans.[14,15] We showed that (1) glycocalyx shedding was generally associated with an increase in plasma heparan sulfate and greater permeability and (2) rats that received blood products (containing plasma) demonstrated full restoration of glycocalyx thickness, permeability, and plasma heparan sulfate to prehemorrhage levels. Heparan sulfate acts as an endothelial cell shear sensor[14] by NOS activation. Hence, the vasodilation mediated by NOS activation requires heparan sulfate as a coligand.[14] In combination with the evidence that microvascular flow is compromised, the differences in heparan sulfate between groups suggest that reconstitution of the glycocalyx structure after hemorrhage may contribute to improved tissue perfusion and endothelial cell protection.

Limitations

This study was limited by the lack of a tissue injury component in this controlled hemorrhage model and because hemorrhagic hypotension could not be allowed to progress to profound shock as reductions in skeletal muscle blood flow would preclude quantitation of in vivo changes in the endothelial glycocalyx, microvascular permeability, and other indices of microvascular function. In addition, our PRBC washing techniques differ from those used clinically to remove plasma "contamination" of the PRBCs and thus fully address the hypothesis.

CONCLUSION

We integrated in vivo microcirculation visualization and clinically-relevant diagnostic measurements to provide data to support the judicious use of blood products in severely wounded soldiers. Plasma protein removal from PRBC concentrates impaired microcirculation and coagulation. PRBC treatment resulted in better-quality microvascular and systemic hemodynamic, metabolic and hemostasis outcomes compared with wPRBC, and restored glycocalyx thickness, microvascular permeability, and coagulation status similar to FWB. Based on previous observations that FFP restored the glycocalyx, further investigation is needed to confirm whether the benefits observed in the present study with PRBC/LR are caused by residual plasma or other RBC effects.

AUTHORSHIP

All authors contributed to the study design. L.N.T. conducted the literature search and data collection. L.N.T. and I.T.F. performed the data analysis. L.N.T., J.L.S., and I.T.F. interpreted the data. L.N.T. wrote the manuscript, which J.L.S., M.A.D., and I.T.F. critically revised.

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DISCLOSURE

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