EVALUATION OF PREHOSPITAL BLOOD PRODUCTS TO ATTENUATE ACUTE COAGULOPATHY OF TRAUMA IN A MODEL OF SEVERE INJURY AND SHOCK IN ANESTHETIZED PIGS

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ABSTRACT—Acute trauma coagulopathy (ATC) is seen in 30% to 40% of severely injured casualties. Early use of blood products attenuates ATC, but the timing for optimal effect is unknown. Emergent clinical practice has started prehospital deployment of blood products (combined packed red blood cells and fresh frozen plasma [PRBCs:FFP], and alternatively PRBCs alone), but this is associated with significant logistical burden and some clinical risk. It is therefore imperative to establish whether prehospital use of blood products is likely to confer benefit. This study compared the potential impact of prehospital resuscitation with (PRBCs:FFP 1:1 ratio) versus PRBCs alone versus 0.9% saline (standard of care) in a model of severe injury. Twenty-four terminally anesthetised Large White pigs received controlled soft tissue injury and controlled hemorrhage (35% blood volume) followed by a 30-min shock phase. The animals were allocated randomly to one of three treatment groups during a 60-min prehospital evacuation phase: hypotensive resuscitation (target systolic arterial pressure 80 mmHg) using either 0.9% saline (group 1, n = 9), PRBCs:FFP (group 2, n = 9), or PRBCs alone (group 3, n = 6). Following this phase, an in-hospital phase involving resuscitation to a normotensive target (110 mmHg systolic arterial blood pressure) using PRBCs:FFP was performed in all groups. There was no mortality in any group. A coagulopathy developed in group 1 (significant increase in clot initiation and dynamics shown by TEG [thromboelastography] R and K times) that persisted for 60 to 90 min into the in-hospital phase. The coagulopathy was significantly attenuated in groups 2 and 3 ($P = 0.025$ R time and $P = 0.035$ K time), which were not significantly different from each other. Finally, the volumes of resuscitation fluid required was significantly greater in group 1 compared with groups 2 and 3 ($P = 0.0067$) (2.8 ± 0.3 vs. 1.9 ± 0.2 and 1.8 ± 0.3 L, respectively). This difference was principally due to a greater volume of saline used in group 1 ($P = 0.001$). Prehospital PRBCs:FFP or PRBCs alone may therefore attenuate ATC. Furthermore, the amount of crystalloid may be reduced with potential benefit of reducing the extravasation effect and later tissue edema.

KEYWORDS—Blood, coagulopathy, plasma, shock, trauma

INTRODUCTION

Trauma is the leading cause of death in the first four decades of life in the developed countries (1, 2), and hemorrhage remains one of the principal causes of death in both civilian and military (battlefield) trauma (3–5). Hemostasis is therefore a key determinant for a patient's survival both immediately after the trauma and over the ensuing hours. Unfortunately a patient's hemostatic potential is impaired by the rapid development of a coagulopathy associated with trauma.

Trauma-induced coagulopathy is now recognized as a serious secondary consequence of injury and the patient's (patho)physiological response to trauma (6–8). Approximately one third of seriously injured civilian casualties (9–11) and potentially similar proportions of military casualties (12, 13) are coagulopathic by the time they arrive in hospital. Trauma-induced

coagulopathy has an evolving pathology in the patient, often starting with the consequences of tissue hypoperfusion and developing through phases that can include the consequences of shock-driven acidosis, hypothermia, iatrogenic (and autogenic) hemodilution, and factor consumption (6, 8). Some authors also suggest an element akin to diffuse intravascular coagulation, without necessarily having tissue hypoperfusion (14). The study reported in this article has targeted the early phase of trauma-induced coagulopathy, associated with tissue hypoperfusion and traumatic shock. This initial phase of trauma-related coagulopathy is referred to as acute trauma coagulopathy (ATC), which manifests as attenuated coagulation early after injury and is associated with tissue hypoperfusion. A number of studies have shown that the presence of ATC is independently associated with increased mortality, morbidity (e.g., organ failure), duration of stay in the intensive care unit, and transfusion requirement (9–11).

The evolving understanding of the mechanism(s) of ATC has to some extent driven therapy. Current therapy centers on early and aggressive use of blood products such as plasma and fibrinogen (6, 8) as well as red blood cells, with an emphasis on a high ratio of plasma to red blood cells, whereas the use of colloids and crystalloids is limited (15). However, our understanding of ATC is far from complete, and resuscitation protocols are still being developed and refined. Volume replacement that augments coagulation function using fresh frozen plasma (FFP),

plasma concentrates, and packed red blood cells (PRBCs) is currently one of the main strategies to treat ATC (6); indeed, there is a long history of this practice in some US civilian hospitals (16) as well as the more consistent drive arising from recent military experience in Iraq and Afghanistan (8). While permissive hypotension has become an established prehospital resuscitation strategy, there is no clear evidence to guide the type and volume of fluid that should be used (6). An empirical approach of administering blood products as early as possible has been adopted clinically. In the majority of cases, this involved early and aggressive in-hospital use of blood products. However, the UK MERT (Medical Emergency Response Teams, UK military helicopter retrieval in Afghanistan) was able to project FFP and PRBCs into the prehospital arena (17). By contrast, civilian helicopter ambulance services in the United Kingdom project PRBCs but no plasma into the prehospital arena (M. Midwinter, personal communication, oral, 1 May 2015). Since prehospital projection of PRBCs and FFP does have significant logistical implications (e.g., ensuring appropriate storage conditions and traceability) and some clinical hazards (e.g., possible transfusion reactions and infection), especially in austere circumstances, it is important to determine whether earlier (prehospital) administration confers advantage compared with immediate in-hospital administration. Furthermore, to aid in the translation of military "lessons learned" into the civilian setting, it is important to compare the military (prehospital PRBCs:FFP) and civilian (prehospital PRBCs alone) putative "best practices" to the original (often current) standard of care (limited use of asanguineous fluid).

The aim of this study was to compare three treatments (PRBCs:FFP, PRBCs alone, and 0.9% saline alone) for simulated prehospital hypotensive resuscitation in a model of complex injury involving both tissue injury and hemorrhagic shock. The prehospital phase was followed by a simulated in-hospital phase, which involved normotensive resuscitation in all cases with PRBCs:FFP (1:1) ratio. The principal outcome variable was coagulopathy assessed using thromboelastography (TEG) 30 min into the in-hospital phase, corresponding to times when surgical decisions are made, often based on the patient's physiological and coagulopathic state. Secondary outcomes included physiological changes and volumes of the various fluids needed for resuscitation, using current standard clinical endpoints based on pressure-driven targets (18, 19).

MATERIALS AND METHODS

The study was conducted on terminally anesthetized cross-bred female Large White pigs (43–56 kg) and was ethically reviewed and conducted in accordance with the Animals (Scientific Procedures) Act, 1986. The animals were housed indoors and were fed on a complete diet comprising coarse ground mixture of wheat, barley, soya protein, vitamins, and minerals. The animals fed *ad libitum* and consumed approximately 5 kg/d. They were allowed water *ad libitum*.

Blood bank
Blood was collected by exsanguination from terminally anesthetised female Large White pigs. Briefly standard units (1 U represents approximately 450 mL) of blood were collected from a carotid cannula at a flow rate of 65 to 90 mL/min into each CPD blood collection bag (RCB434CCL; Pall Medical, Portsmouth, UK), with pauses between bag collection resulting in an overall bleeding rate of 22.5 mL/min. The blood was processed according to standard UK blood transfusion protocols (20) to separate the red blood cells from the plasma, which was leukodepleted, whereas the PRBCs were stored with SAG-M. The platelets

were discarded. Collected units of blood were processed within 90 min of collection. The resulting units of PRBCs were stored at 4°C (LabCold Blood Bank, Basingstoke, UK) and used within 14 days of collection. The plasma (FFP) was fast-frozen (MP1100 Plasma Freezer System; Thermogenesis, Noblesville, US), stored at −30°C (LabCold Plasma Freezer; LabCold), and used within 6 months of collection. Fresh frozen plasma was thawed at 37°C in a dry plasma thawer (Sahara III Maxitherm; Sarstedt, Nümbrecht, Germany) immediately before use. Prior to use, all donor products were forward and reverse matched to recipient blood. In addition, because PRBCs and FFP from different donors were used for resuscitation, they were also cross-matched with each other.

Surgical preparation
The animals were fasted for 18 h before the surgical procedure, but allowed water *ad libitum*. After premedication with intramuscular midazolam hydrochloride (0.1 mg/kg), anesthesia was induced by mask with isoflurane (5%) in a mixture of oxygen and nitrous oxide (1:1), and the animals intubated. Surgical anesthesia was subsequently maintained with isoflurane (1%–2%) in a mixture of oxygen and nitrous oxide (1:2), and the animals ventilated using positive-pressure ventilation (Blease Manley MP3 Anaesthesia Ventilator). Initial monitoring consisted of end-tidal CO_2, pulse oximetry through a tail probe, and skin surface electrocardiogram electrodes (Propac 106EL; Protocol Systems Inc, Beaverton, Ore). With the animal positioned supine, surgical preparation took place after skin preparation with povidone-iodine solution (10% wt/vol, Betadine Aqueous Antiseptic Solution; Seaton Healthcare Group plc, UK).

The left carotid artery (Swan Ganz; Edwards Lifesciences Ltd, Newbury, UK), both internal jugular veins, and left femoral artery and vein were cannulated (Portex 8FG; Sims Portex Ltd, Hythe, UK). A balloon-tipped flow-directed cannula (744MF75 Swan Ganz; Edwards Lifesciences Ltd) was introduced through a right internal jugular vein cannula introducer sheath (Desivalve Catheter Introducer; Vygon, Cirencester, UK) and advanced until its tip was in the pulmonary artery. Cannula placement was determined by monitoring pressure changes at the tip.

After venous access had been established, anesthesia was continued with intravenous alphaxalone to maintain a surgical plane of anesthesia assessed by palpebral reflex and jaw tone (Alfaxan; Jurox (UK) Ltd, Malvern Link, UK), and the isoflurane discontinued.

A midline laparotomy was performed, the spleen contracted by topical application of adrenaline (up to 1.5 mL of a 1mg/mL solution) before removal, and the bladder catheterized by open suprapubic cystostomy. All incisions were closed *en masse*. Animals were allowed to breathe spontaneously for the remainder of the experiment unless they displayed marked respiratory depression, at which stage synchronized intermittent mandatory ventilation (Drager Evita 2; Draeger Medical UK Ltd, Hemel Hempstead, UK) was instituted in an attempt to maintain adequate oxygenation and prevent severe hypercapnea. The animals recovered from surgery under anesthesia for 1 h before baseline measurements were made.

Cardiovascular monitoring
Arterial blood pressure was recorded through the carotid artery cannula, and pulmonary arterial and central venous pressures were recorded via the flow-directed, balloon-tipped flotation catheter, which was also used to determine cardiac output as a 6-min rolling average (Vigilance Volumetrics CEDV; Edwards Lifesciences Ltd). Physiological pressure measurements were made using strain gauge manometers (Sensonor 840; SensoNor a.s., Skoppum, Norway), and zero pressure for all transducers was set at heart level. Body temperature was maintained at approximately 38°C using external heating/cooling and blankets as appropriate. The bladder was drained at hourly intervals.

All cardiovascular variables were recorded using a computerized data acquisition system (Maclab 8/s; ADInstruments, Oxford, UK) and associated software (Chart v4.2.3; ADInstruments) for subsequent analysis.

Blood gas and related chemistry
Arterial and venous blood samples were taken anaerobically into heparinized syringes from the carotid and pulmonary artery catheters, respectively, for blood gas, base excess, and lactate analysis (Gem Premier 3000 Blood Gas Analyzer; Instrumentation Laboratories, Warrington, UK).

Experimental protocol
The animals were randomly allocated to one of three treatment groups at the outset (Fig. 1). One hour after the end of surgery, three cardiovascular measurements were made 5 min apart and paired arterial and mixed venous blood gas samples taken at the time of the first and third baseline cardiovascular measurement. The timeline for the experimental protocol is shown in Figure 1.

Injury phase—All animals were subjected to a controlled soft tissue injury using a blunt captive bolt pistol (CASH Special Knocker; Accles & Shelvoke,
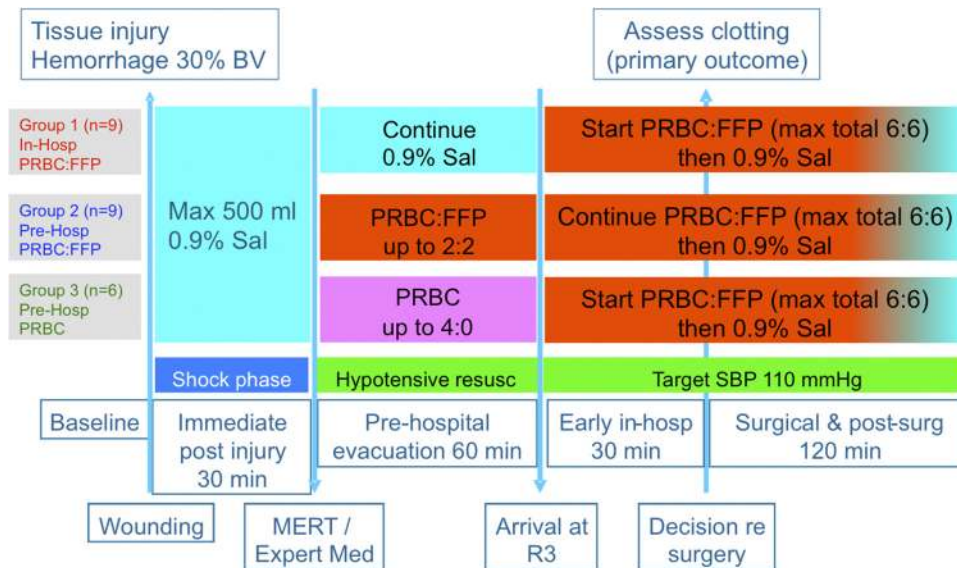
Fig. 1. **Experimental protocol commencing approximately 1 h after the end of surgery showing the phases of the experimental protocol and corresponding clinical phases for three groups of animals (treatment groups 1–3 that diverge during the simulated evacuation phase where casualties may be treated by the Medical Emergency Response Teams to receive expert medical attention, Expert Med before arrival at a field hospital, R3)**. In-hospital (In-Hosp) phase divided into early and later surgical and postsurgical separated by assessment of primary outcome (clotting using TEG), treatment identical in both in-hospital phases. BV indicates total estimated blood volume; Sal, saline. Ratios indicate ratio of PRBCs to FFP and indicates capped amount in units (approximately 450 mL) of each constituent.

Sutton Coldfield, UK) delivering four standard impacts (using 2 Grain .25 cartridges) to the muscle of the right hindquarter. This resulted in widespread deep contusion in the underlying muscle but no fracture to bone. Five minutes after the tissue injury, a controlled hemorrhage of 35% of the estimated total blood volume was performed over 9 min 40 s via the femoral arterial cannula, using a computer-controlled pump (Masterflex L/S model 7550-17; Cole Palmer Instrument Company, Chicago, Ill). The rate of bleeding reduced exponentially as the hemorrhage progressed to mimic the rate of hemorrhage from a major arterial lesion. For blood volume and bleeding rate equations, see Garner et al. and Stern et al. (21, 22).

Shock phase—Following hemorrhage, the animals underwent a 30-min shock period during which a capped volume of 0.9% saline (500 mL maximum) was administered as necessary to maintain a target systolic arterial blood pressure (SBP) of 60 mmHg, reflecting an aspect of current clinical practice by combat medical technicians (19). In addition, this was found to be necessary in a pilot phase to avoid mortality in some animals. The practice was standardized by applying a predefined and clinically relevant target to all animals. There were no significant differences in the volumes of saline given in the three groups in this phase of the study ($P = 0.3450$), which are shown in Figure 10. Resuscitation infusions were warmed to 37°C and administered at a rate of 200 mL/min (Belmont Rapid Infuser; Belmont Instrument Corporation, Billerica, Mass).

Prehospital evacuation phase—The next phase of the protocol represented a 60-min prehospital evacuation phase. Warmed resuscitation fluid was administered according to the relevant protocol, with a final target SBP of 80 mmHg in each group. Group 1 (n = 9) was given aliquots of 0.9% saline (representing standard of care) to attain and maintain the target SBP, whereas group 2 (n = 9) received PRBCs and FFP (1:1 ratio, PRBCs and FFP given simultaneously), and group 3 (n = 6) was given PRBCs only. The volume of blood products in the prehospital phase was capped at 4 U (4 × 450 mL approximately) per animal (2 U PRBCs and 2 U FFP in group 2, and 4 U PRBCs in group 3, respectively, reflecting current military and civilian prehospital emerging practice in the United Kingdom). Once the maximum amount of blood product had been used, resuscitation continued to the same pressure target using 0.9% saline for the reminder of the prehospital evacuation phase. All infusion volumes were measured accurately.

In-hospital phase—The in-hospital phase represented more aggressive resuscitation to a normotensive target (SBP 110 mmHg) using PRBCs:FFP (1:1) in all groups. Supplementary oxygen (minimum F_{IO_2} 0.3) was given and titrated to attain an arterial oxygen saturation (S_{aO_2}) of 98%. Because of the limitations of the blood bank, the total amount of PRBCs:FFP used in any one animal was capped at 6 U of each product (in addition to the PRBCs used during the prehospital phase in group 3). Once the total permissible amount of PRBCs:FFP had been given, resuscitation continued to the same pressure target using 0.9% saline in all groups. In practice, few animals received saline in the in-hospital

phase (three of nine in group 1; two of nine in group 2; none of six in group 3; Fig. 10). All animals in this study survived to the end of the protocol.

Sampling and measurements

Cardiovascular and paired arterial and mixed venous blood gas measurements were made before and after injury/hemorrhage, after 15- and 30-min shock, and at 30-min intervals during the prehospital resuscitation phase and in-hospital resuscitation phases. Blood samples for assessment of clotting were also taken at these timepoints.

Coagulation assays

Thromboelastography—Thromboelastography using a TEG 5000 Hemostasis Analyzer (Haemonetics Ltd, Coventry, UK) was performed on fresh, uncitrated whole blood. Arterial blood was taken from the femoral cannula and analyzed immediately using dilute Innovin (1:50,887 dilution Dade Innovin; Dade Behring, marketed by System UK Ltd, Milton Keynes, UK) as the initiator (23). All TEG analyses were performed in triplicate at 37°C.

Prothrombin and activated partial thromboplastin times—Arterial blood samples were taken into citrated Vacutainers (9NC 0.105 M Vacutainer 367691; Becton Dickinson, Oxford, UK) and centrifuged at 1,500g for 10 min. The plasma was separated and stored at −80°C for determination of prothrombin time (PT) and activated partial thromboplastin time (aPTT) by turbidometry and fibrinogen concentration (Clauss method) using an ACL Elite analyzer (Instrumentation Laboratories).

Study endpoints

The primary endpoint of the study was the clotting status assessed using TEG after 30 min of in-hospital resuscitation. Secondary endpoints included clotting status throughout the study and physiological status, in particular degree of shock assessed by measuring actual base excess (ABE) and arterial lactate and volumes of fluids used to attain the pressure-driven resuscitation targets.

At the end of the study, all animals were killed humanely using a lethal overdose of sodium pentobarbitone (Euthatal; Merial Animal Health Ltd, Harlow, Essex, UK) given intravenously.

Statistical analysis

All data are presented as mean ± SEM unless indicated otherwise. Data were assessed for normality and subjected to transformation if necessary. Time series data were analyzed using 2-way analysis of variance (ANOVA) with repeated measures over time. Single timepoint analyses were made using 1-way ANOVA with Fisher comparison of means for planned between-group comparisons. Where it was not possible to conduct a parametric analysis, a Kruskal-Wallis

Table 1. **Initial values of physiological data and body weights**

	Group 1	Group 2	Group 3	P
No. animals	9	9	6	
Pao$_2$, kPa	8.9 ± 0.1	9.2 ± 0.2	8.4 ± 0.4	0.101
Paco$_2$, kPa	6.6 ± 0.2	6.6 ± 0.2	6.8 ± 0.2	0.805
Art pH	7.44 ± 0.01	7.43 ± 0.01	7.44 ± 0.01	0.496
Hct, %	40.8 ± 0.6	41.9 ± 0.9	41.8 ± 0.7	0.481
Fibrinogen, g/L	2.6 ± 01	2.3 ± 0.1	2.6 ± 0.2	0.171
Body temperature, °C	39.1 (0.4)	38.8 (0.8)	38.2 (1.2)	0.030*
Body weight, kg	51.0 (4.5)	53.0 (6.0)	53.0 (4.5)	0.891*

Body weights and initial values of arterial oxygen and carbon dioxide tensions (Pao$_2$ and Paco$_2$) and pH (Art pH), hematocrit (Hct), and body temperature.
P value for difference between groups.
*Comparison performed using Kruskal-Wallis one-way ANOVA because data were not normally distributed. Values are mean ± SEM or median (interquartile range).

multiple-comparisons test was used followed by Dunn test for planed between-group comparisons. Any other tests are indicated in the text. Statistical analysis was performed using NCSS Statistical Software (NCSS, LLC, East Kaysville, Utah). In all cases, a significance level of $P < 0.05$ (two-tailed) was used.

RESULTS

All animals survived to the end of the study. Baseline (preinjury) values are shown in Table 1. There were no significant differences between groups in the initial parameters except for body temperature; although the difference between groups was statistically significant, they are unlikely to be of physiological consequence. Body temperature did not change significantly during the course of the study ($P = 0.3560$).

Cardiovascular effects of tissue injury, hemorrhage, and resuscitation

Tissue injury and hemorrhage led to a significant fall in arterial blood pressure and cardiac index in all groups ($P < 0.001$). There were no significant differences in the cardiovascular response to hemorrhage between groups ($P = 0.243$; Fig. 2). Systolic arterial blood pressure was thereafter maintained at the relevant target for each phase of the protocol (Fig. 1). The

targets were attained in all groups, resulting in significant elevations in blood pressure between phases ($P < 0.001$; Fig. 2).

Effects on clotting

Thromboelastography—The effect of the prehospital treatments on the primary outcome of the study is summarized in Table 2. At the onset of the in-hospital phase (after 60 min of resuscitation), there were significant differences between groups. This was due to group 1 (that had hitherto received 0.9% saline only in the prehospital phase) displaying markedly elevated R and K times compared with both groups 2 and 3. There were no significant differences between groups 2 and 3. A similar situation was still present 30 min into the in-hospital phase, which was the primary outcome of this study.

Time series data for the whole study showing overall changes in coagulation are shown in Figure 3. Combined injury and hemorrhage led to a small reduction in R and K times ($P = 0.0661$ and $P = 0.0182$, respectively). There were no significant differences between groups during the entire baseline, injury/hemorrhage, and shock phases for either R or K times ($P = 0.6566$ and $P = 0.6977$, respectively; Fig. 3). During the prehospital phase, there were very marked alterations in both R and K times leading to significant differences between groups ($P = 0.0098$ and $P = 0.0027$). By the end of the prehospital phase (60 min after the start of resuscitation), there was a clear, significant difference between groups in both R and K times ($P = 0.006$ and $P = 0.002$, respectively; Figure 3 and Table 2).

Overall, during the in-hospital phase, there were significant differences between groups in R and K times (Fig. 3 and Table 2), significant changes over time ($P = 0.0019$ and $P = 0.0451$), and significant differences in patterns of response between groups ($P < 0.0001$ and $P = 0.0027$). By 150 min after the onset of resuscitation (90 min of in-hospital resuscitation), both R and K times had recovered in group 1 to levels that were not significantly different to those seen in the other two treatment groups ($P = 0.079$ and $P = 0.243$). Therefore, group 1 developed a coagulopathy that was apparent during the first hour of in-hospital resuscitation with PRBCs:FFP and subsequently resolved. By contrast, clotting in both groups 2 and 3 was stable throughout.
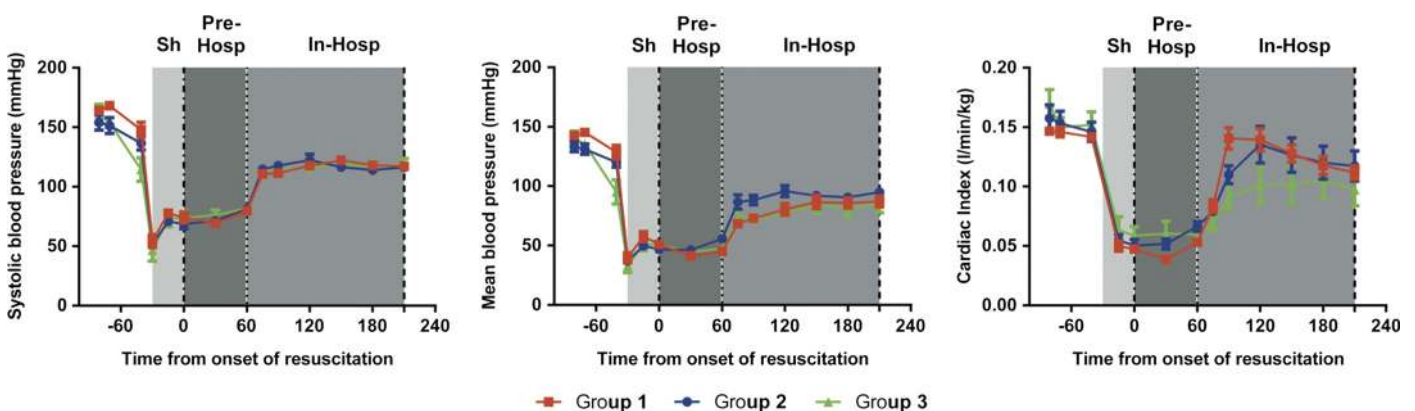

Fig. 2. **Effects of issue injury and hemorrhage, followed by a shock phase (Sh), prehospital hypotensive resuscitation phase (Pre-Hosp), and in-hospital normotensive resuscitation phase (In-Hosp)**. Treatment groups differed in the fluid used for resuscitation during Pre-Hosp; group 1 (n = 9) received 0.9% saline, group 2 (n = 9) received 1:1 PRBCs and FFP, and group 3 (n = 6) received PRBCs without FFP. All groups received PRBCs:FFP (1:1) during the in-hospital phase. The first three datapoints represent, respectively, baseline value followed immediately before and immediately after tissue injury and hemorrhage. Systolic and mean arterial blood pressures (SBP and MBP) and cardiac index (CI). Mean values ± SEM.

TABLE 2. **Primary outcome measure (clotting assessed by TEG after 90 min of resuscitation, corresponding to 30 min in-hospital)**

Timepoint	TEG parameter	Group 1 prehospital saline	Group 2 prehospital PRBCs:FFP	Group 3 prehospital PRBCs alone	Statistical P			
					Between groups	G1 vs. G2	G1 vs. G3	G2 vs. G3
60-min Resuscitation (immediate in-hospital)	R, min	40.4 ± 8.8	17.4 ± 2.9	14.7 ± 1.9	0.006	0.004	0.007	0.851
	K, min	17.1 ± 3.4	6.8 ± 1.5	5.8 ± 0.9	0.002	0.002	0.003	0.900
	MA, mm	68.1 ± 2.6	65.8 ± 2.1	64.7 ± 1.5	0.587	NA	NA	NA
90-min Resuscitation (30 min in-hospital)	R, min	38.3 ± 10.7	16.9 ± 2.4	14.5 ± 1.7	0.025	0.017	0.022	0.887
	K, min	19.1 ± 6.2	6.1 ± 1.5	5.6 ± 0.9	0.035*	<0.05	<0.05	>0.05
	MA, mm	60.8 ± 5.0	64.8 ± 2.0	65.1 ± 1.5	0.586	NA	NA	NA

Data are also shown for clotting after 60 min of resuscitation (corresponding to arrival at hospital).
*Comparison performed using Kruskal-Wallis one-way ANOVA on ranks followed by Dunn comparisons because the data were resistant to transformation and could not be analyzed using a parametric test.
NA indicates further comparisons not applicable because there is no overall difference between groups. Values are mean ± SEM.

In the model used in this study, clot strength, assessed using TEG maximum amplitude (MA; Fig. 3), showed a significant increase during the injury/hemorrhage and shock phase ($P = 0.014$), but no difference between groups ($P = 0.319$). Subsequently, there was a small fall in MA, but no change indicative of a coagulopathy in any group. No significant difference in MA was found between groups at any time during the study.

Activated partial thromboplastin time and PT—Activated partial thromboplastin time (Fig. 4) showed a similar pattern to that reported for TEG R and K times. However, there were significant differences in baseline aPTT between groups ($P = 0.023$); therefore, all subsequent analyses were performed by determining changes from baseline to eliminate the effects of baseline differences. During the baseline, injury/hemorrhage, and shock phases, there were no significant differences between groups ($P = 0.9655$). However, during the prehospital phase, there was a significant change in aPTT ($P = 0.0011$), and a different pattern between groups ($P = 0.0018$), with a marked increase in aPTT in group 1, but not in the other two groups. By the in-hospital phase (60 min from the start of resuscitation), aPTT was higher in group 1, although this did not attain statistical significance ($P = 0.0818$). Overall, there was a significant change in aPTT over time during the in-hospital phase ($P = 0.0002$), and again a different pattern between groups ($P = 0.0466$), with aPTT in group 1 recovering toward levels seen in the other two groups in the latter part of the in-hospital resuscitation phase.

The initial changes in PT were similar to those seen in aPTT (shock and prehospital resuscitation phases); however, the pattern then differed markedly in the in-hospital phase (Fig. 4). During the baseline, injury/hemorrhage, and shock phase, there was no difference in PT between groups ($P = 0.2038$), although there was a small but statistically significant change over time ($P < 0.001$). During the prehospital resuscitation phase, there were significant differences between treatment groups ($P = 0.0054$) and over time ($P < 0.0001$) as PT rose in group 1, but not in the other two groups ($P = 0.0011$). By the end of the prehospital phase, PT was significantly higher in group 1 compared with groups 2 and 3 ($P = 0005$ and $P = 0.0251$). During the in-hospital phase, the significant difference between groups persisted ($P = 0.0203$). There was a change in PT over time in the in-hospital phase ($P < 0.001$), but no difference in pattern between groups ($P = 0.3057$). Therefore, PT data showed a coagulopathy developing in the group 1 when compared with group 2 during the prehospital and early in-hospital resuscitation phases, but in contrast to the other parameters (TEG and aPTT), this did not resolve in any group during the latter part of the in-hospital phase (Fig. 4).

Fibrinogen levels and platelet count—There were no significant differences in baseline fibrinogen levels between groups ($P = 0.172$). During the injury/hemorrhage and shock phases, there was a statistically significant fall in fibrinogen levels ($P = 0.001$) without any differences between groups
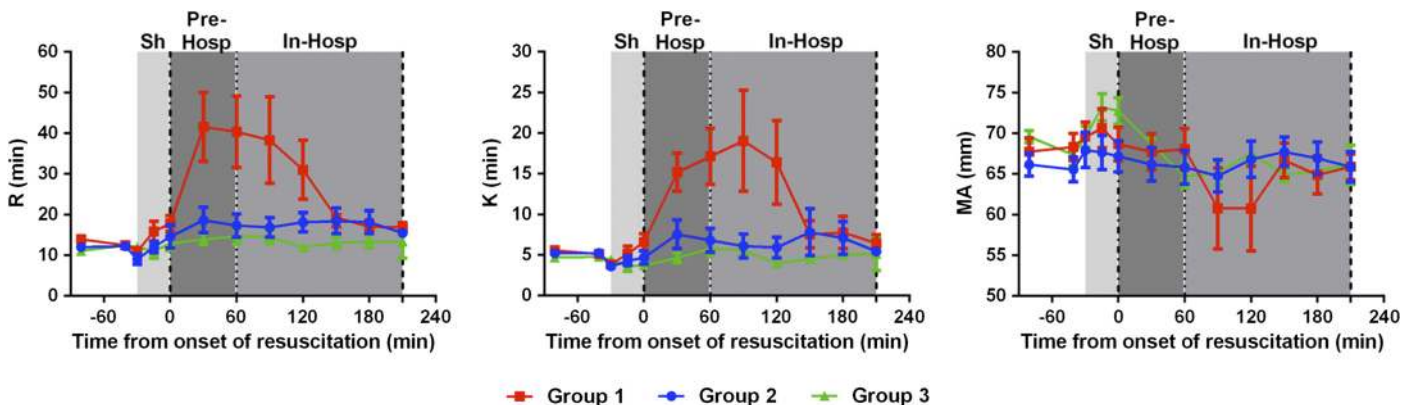

FIG. 3. **Effects of tissue injury, hemorrhagic shock, and resuscitation on TEG R time (clot initiation), K time (clot dynamics), and MA (clot strength) in three treatment groups**. For more details, see legend to Figures 1 and 2. Mean values ± SEM.
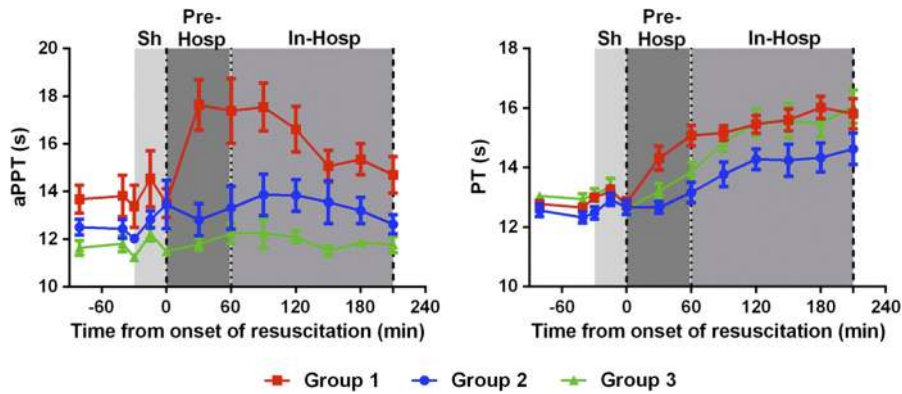
FIG. 4. **Effects of tissue injury, hemorrhagic shock, and resuscitation on aPTT and PT in three treatment groups**. For more details, see legend to Figures 1 and 2. Mean values ± SEM.

($P = 0.99$; Fig. 5), although this is unlikely to be of clinical significance. During the prehospital phase, fibrinogen fell further in all groups ($P = 0.002$), and again there were no statistically significant differences between groups ($P = 0.154$). By the end of prehospital resuscitation, fibrinogen was lowest in group 1, although this was not statistically different from the other groups (Fig. 5). The lowest mean level attained in group 1 (1.50 ± 0.12 g/L) was only slightly below the 95% reference range for this strain of pigs (1.77–3.36 g/L, n = 147). (Data based on samples taken after minimal surgery [induction of anesthesia and insertion of carotid cannula] in Large White pigs of similar age that contributed to this and other studies in our laboratory. Data distribution was normalized with a 1/square root transformation prior to determining the reference range.) Seven of nine pigs in group 1 were below the reference range at the end of prehospital resuscitation compared with four of nine in group 2 and four of six in group 3.

The fall in fibrinogen levels at the end of the prehospital phase was due to more than the hemodilution associated with resuscitation. Changes in hematocrit (Fig. 5) were taken as a marker of hemodilution during the prehospital phase in group 1 because there was no external gain or loss of red blood cells during this phase in group 1. The ratio of the proportional fall in fibrinogen levels from baseline to the proportional fall in hematocrit from baseline should be 1 if the reduction in fibrinogen levels is in proportion to hemodilution and below one if there is additional loss of fibrinogen. The ratio at the end of the

prehospital phase in group 1 was 0.84 ± 0.04, which was significantly below 1 ($P = 0.009$, one-sample t test), suggesting loss of fibrinogen over and above the effects of hemodilution. During the subsequent in-hospital phase, there was a small elevation in fibrinogen levels that was statistically significant ($P = 0.005$), but no significant difference between groups ($P = 0.332$).

There was a fall in platelet count in all groups over the course of the study, which attained statistical significance ($P = 0.001$; Fig. 5). However, there was no statistically significant difference between groups at any stage in the study ($P = 0.423$).

Blood chemistry and oxygen transport

Arterial base excess, lactate, and shock—The degree of shock was evaluated by measuring changes in arterial base excess and lactate levels (Fig. 6). During hemorrhagic shock, there was a marked clinically and statistically significant fall in arterial ABE ($P < 0.0001$) and a rise in arterial lactate ($P < 0.0001$), without any significant difference between groups ($P = 0.7787$ and $P = 0.8300$, respectively). This was associated with a significant elevation in whole-body oxygen extraction ratio (OER; Fig. 7) from normal preinjury baseline levels in the range 0.25 to 0.30 to theoretical maximal levels in the range 0.78 to 0.80 ($P < 0.0001$). There were no significant differences in OER between groups ($P = 0.254$; Fig. 7). The high levels of OER persisted in all groups throughout the prehospital phase, whereas base excess continued to fall and lactate rose ($P < 0001$ and $P < 0.0001$). There was a trend for
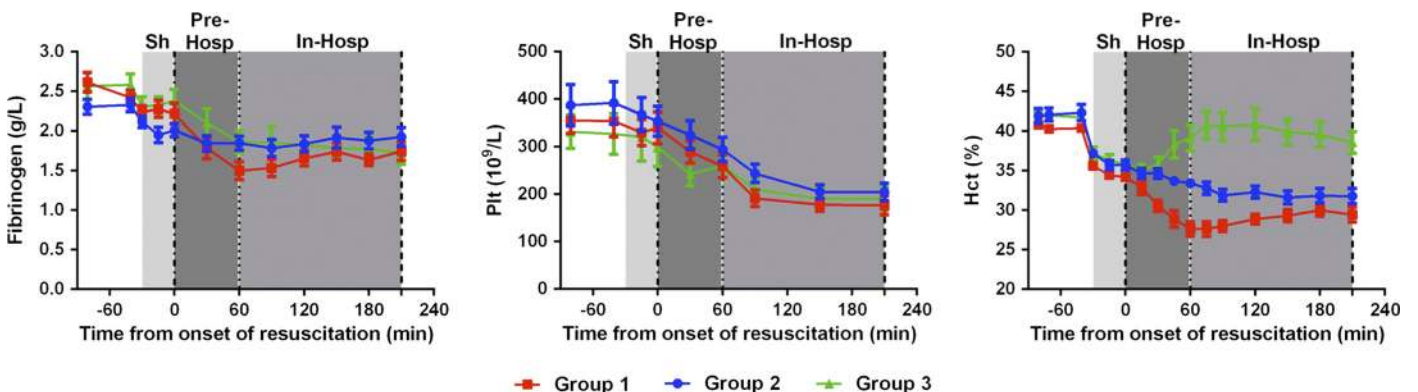

FIG. 5. **Effects of tissue injury, hemorrhagic shock, and resuscitation on fibrinogen levels, platelet count (Plt), and hematocrit (Hct) in three treatment groups**. For more details, see legend to Figures 1 and 2. Mean values ± SEM.
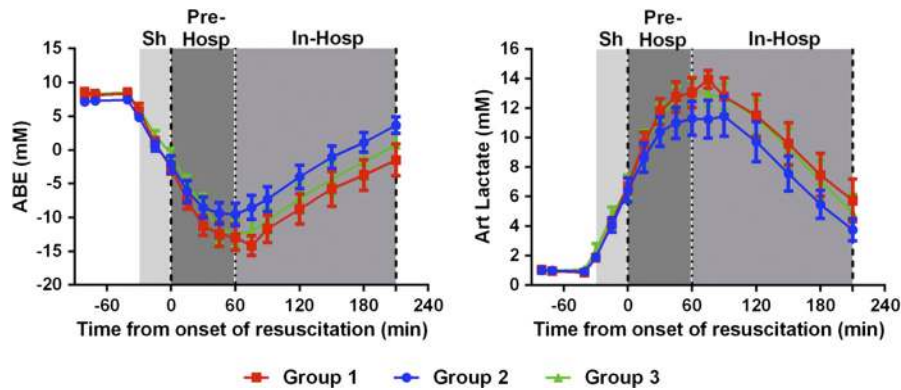
FIG. 6. **Effects of tissue injury, hemorrhagic shock, and resuscitation on arterial base excess (ABE) and lactate (Art Lactate) in three treatment groups**. For more details, see legend to Figures 1 and 2. Mean values ± SEM.

ABE to reach its nadir and lactate to reach its peak in group 2, whereas these variables continued to deteriorate in the other two groups; however, there were no significant differences between groups for either ABE ($P = 0.3937$) or lactate ($P = 0.4330$). Throughout the in-hospital phase, the trend for less negative ABE and lower lactate in group 2 persisted, although again this was not statistically significant ($P = 0.1483$ and $P = 0.4720$, respectively).

The cumulative burden of shock was evaluated by determining the area under the ABE curve for each individual pig (Fig. 8). The area under the curve was significantly less in group 2 when compared with group 1 ($P = 0.0380$, Mann-Whitney U test). It was not possible to compare group 3 to the other two groups because of the difference in distribution of data in group 3 that proved resistant to transformation and precluded statistical comparison.

Oxygen transport—Hematocrit fell significantly in all groups during the shock phase ($P < 0.0001$), with no difference between groups ($P = 0.1420$). Once the treatment groups diverged in the prehospital phase, differences between groups became significant ($P < 0.0001$), with hematocrit rising in group 3 and falling in the other two groups. The greatest fall was in group 1. Hematocrit remained significantly higher in group 3 compared with the other two groups for the reminder of the study, whereas groups 1 and 2 converged during the in-hospital phase. Arterial oxygen content followed the same pattern (Fig. 7). There was a significant fall in mixed venous oxygen content during the

shock and prehospital phases ($P < 0.0001$), followed by an increase during the in-hospital phase (Fig. 7, $P < 0.0001$). There were no differences between groups in mixed venous oxygen content at any stage in the study ($P = 0.099$).

Fluid administration

The cumulative volumes of combined fluid given during the study are shown in Figure 9. There was no significant difference between groups in the volume of saline required during the shock phase (Fig. 10, $P = 0.3450$). Overall, group 1 required a significantly greater volume of combined fluids during the prehospital phase and over the course of the study ($P = 0.0005$ and $P = 0.0067$; Fig. 10). The increased combined fluid requirement in group 1 was due to a significantly greater volume of saline used in this group compared with the other two ($P = 0.0001$, Kruskal-Wallis multiple-comparisons test). Most of this saline was administered in the prehospital phase. There was no overall difference in the amount of PRBCs:FFP required between the groups (Fig. 10, $P = 0.2748$).

DISCUSSION

In this study, we modeled severely injured casualties who require resuscitation to sustain life *en route* to hospital. The model incorporated tissue injury, controlled hemorrhage, and hemorrhagic shock followed by resuscitation to clinically relevant targets, representing prehospital and in-hospital phases
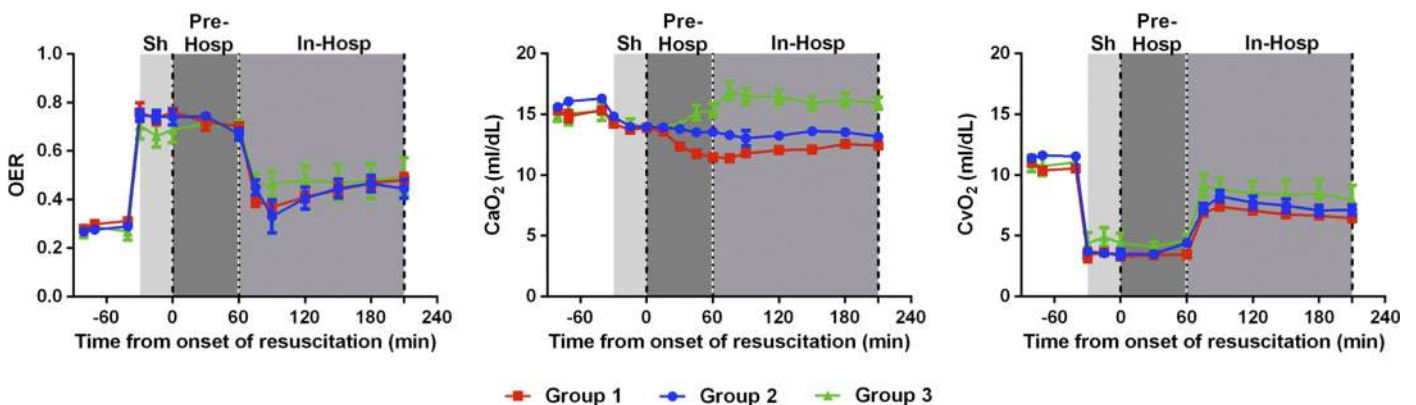

FIG. 7. **Effects of tissue injury, hemorrhagic shock, and resuscitation on OER, arterial oxygen content (CaO_2), and mixed venous oxygen content (CvO_2) in three treatment groups**. For more details, see legend to Figures 1 and 2. Mean values ± SEM.
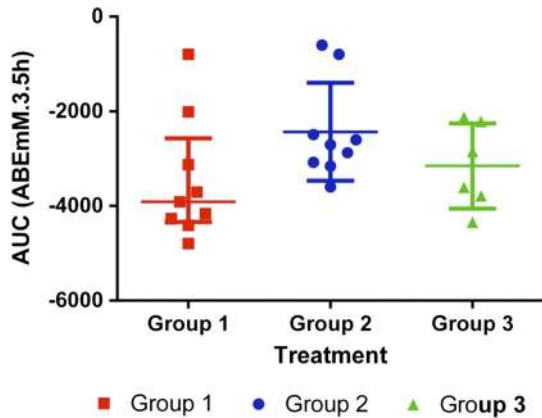
FIG. 8. **Cumulative area under the arterial base excess curve for each individual animal (AUC)**. Area integrated between start of resuscitation (beginning of prehospital resuscitation) and end of the in-hospital phase. Negative values indicate shock. Data shown for each individual animal in three treatment groups, and medians and interquartile ranges.

of treatment, which resulted in 100% survival to the end of the study in all groups presented in this report. The principal finding is that use of blood products for "prehospital" resuscitation attenuated the coagulopathy that developed in the control group (group 1), which was given 0.9% saline only in the prehospital phase. A surprising finding was that the use of PRBCs alone in the prehospital phase was as effective as PRBCs:FFP (1:1 ratio) in avoiding ATC in this model. This finding is consistent with the concept that it is tissue hypoperfusion and possibly reduced oxygen delivery that are the early drivers of ATC (24, 25). More aggressive "in-hospital" resuscitation with PRBCs:FFP resolved the coagulopathy in group 1 after a delay of approximately 60 min, and by 90 min in-hospital the coagulation parameters had converged in all the groups. In our model, therefore, prehospital use of blood products does confer benefit extending into relevant in-hospital phases, even when aggressive resuscitation strategies are used from the outset in the in-hospital phase.

Saline 0.9% was chosen as the control limb in this study principally because it is the most commonly used prehospital resuscitation fluid by UK forces and is currently in widespread use in civilian practice despite concerns expressed by some about its "unphysiological" effects. The purpose of this study was to compare emerging treatment with current clinical standards; hence, colloid solutions were not used simply because they are not in widespread prehospital use for trauma casualties. This is despite their having the scientific merit of more closely resembling plasma with respect to retention in the vascular space. Alternatives such as starch solutions (e.g., Hextend) were not used in the present study because they are used less commonly in the prehospital arena (e.g., 36% of US casualties in Iraq compared with 64% given crystalloid [26]), and in the case of starch solutions, they may impair coagulation by inducing additional hemodilution and by direct interaction with clotting mechanisms (26).

Fibrinogen was found to decrease in all groups during shock and subsequent resuscitation, with the greatest fall being seen in group 1. It is difficult to determine the contribution of this fall in fibrinogen levels to the coagulopathy developed in group 1 during prehospital resuscitation. At the end of the

prehospital phase, the fibrinogen level in group 1 was slightly below the 95% reference range for this strain of pigs; however, this is in the context of a shock state and reduced platelet count, which may have accentuated the effects of the lowered fibrinogen. In the other two (blood products) groups, fibrinogen levels fell to mean levels that were slightly above the lower 95% reference range for the pigs. The most likely conclusion is that the fall in fibrinogen levels may have contributed to the coagulopathy seen in group 1, but it is unlikely to be the sole or even major contributor to the difference between groups.

Secondary findings relate to changes in physiological state and volumes of fluid used for resuscitation. All groups developed a significant degree of shock during the prehospital phases, seen as a marked fall in base excess and elevated lactate levels. Prehospital PRBCs:FFP (group 2) appeared to afford the greatest protection against shock, which is counterintuitive because, predictably, arterial oxygen content was highest in group 3 (given prehospital PRBCs alone). Unfortunately, it was not possible to compare statistically the cumulative burden of shock between groups 2 and 3 because of the different distribution in the two groups, but the overall levels of both base excess and lactate in group 3 are nearer group 1 than group 2 (Fig. 6). It is clear that oxygen extraction was maximal in all of the groups during the prehospital phase (equally high OER and low mixed venous oxygen content in all groups), suggesting that the low blood flow state inherent in hypotensive resuscitation was the limiting factor. It is possible that the better performance of PRBCs:FFP may reflect a beneficial effect of the plasma on the microvasculature, possibly via an action on the endothelium and/or glycocalyx (27–29), which optimized local control and oxygen delivery. In addition, the buffering capacity of FFP may also have played a part in reducing the acidosis.

The overall volume of fluid needed for resuscitation was considerably less in the groups given prehospital blood products. This difference was due to a much greater volume of saline needed in group 1, and the magnitude is likely to be of clinical significance especially given the emerging concerns regarding the deleterious effect of crystalloid on the vascular endothelium and inflammatory responses (30). In a military context, the reduction in volume of prehospital fluid requirement is
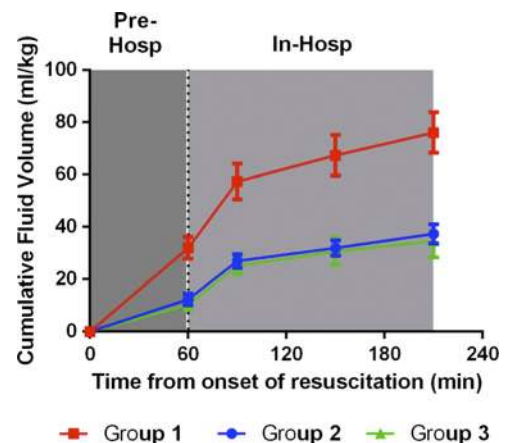

FIG. 9. **Cumulative total of combined resuscitation fluid infusion (0.9% saline, PRBCs:FFP, and PRBCs alone) in three treatment groups**. For more details, see legend to Figures 1 and 2. Mean values ± SEM.
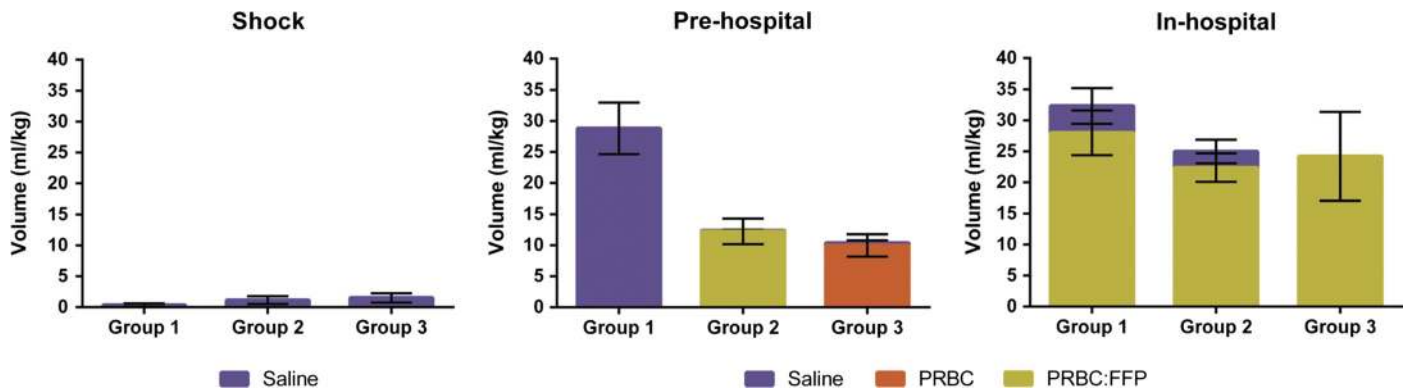
Fig. 10. **Total volume of component fluids given in each phase of the protocol (shock, prehospital resuscitation, and in-hospital resuscitation) in three treatment groups**. Mean values ± SEM.

likely to have a logistical benefit, although the cooling required for blood products to some degree negates this. Future studies will need to evaluate products that confer the physiological benefits of PRBCs and FFP without the logistical burden of refrigeration. Lyophilized plasma has already shown promise in both animal studies (31) and emerging clinical practice (32–34). Finally, it was reassuring that the early administration of blood products did not increase the overall demand for these products and is therefore unlikely to result in a greater overall burden on blood banks.

The results of our study are in broad agreement with a recent observational clinical study that compared prehospital transport platforms, one of which used plasma and red blood cells (1:1), and the other(s) used crystalloids (35). This observational study reported a statistically significant improvement in physiological status (base excess, −3 vs. −4 mM) and a clear reduction in volumes of prehospital fluids needed (35). However, there was no difference in measured clotting status between groups in this study (35). One possible explanation for this is that although the patients were severely injured based on the Injury Severity Scores, the degree of underlying shock was not great, in contrast to our study where there was a more profound degree of shock. Although our study shows that prehospital PRBCs alone was also effective in reducing coagulopathy, these results should not be taken to contradict the clinical trials (such as the PROPPR trial [36]) that show high ratios of plasma to red blood cells provide benefit such as improved hemostasis. The most likely explanation for the beneficial effect of PRBCs alone in our study rests with the evolving mechanism underpinning ATC (37), such that the very early phase may indeed be responsive to improved perfusion and oxygen delivery, although the contribution of any residual plasma in our PRBCs cannot be ruled out.

In any experimental study, the nature of the model used defines the limitations of the conclusions. In this study, we were attempting to model a severely injured battlefield casualty with a significant burden of injury and hemorrhagic shock, who would initially receive very limited resuscitation (19, 38, 39) once hemorrhage has been arrested, prior to evacuation to a hospital. Our model therefore incorporated elements of significant tissue injury and hemorrhagic shock (with associated hypoperfusion) as suggested by Frith et al. (40), followed by clinically relevant resuscitation, all in the correct chronological

sequence. A 1-h evacuation timeline was selected because it is broadly representative of recent military (38, 41) and civilian evacuation timelines in the United Kingdom and Europe (9, 10) and United States (35) during which hypotensive resuscitation is practiced (18, 19), although shorter timelines have been reported in a number of US studies (24, 25). The endpoint of resuscitation was a pressure-driven target because this is all that there is currently available in the prehospital military setting, i.e., administration of fluid to attain a palpable carotid (corresponding approximately to a SBP of 60 mmHg) or radial (SBP 80 mmHg) pulses. To mimic the current clinical practice, fluid was therefore administered only if arterial blood pressure fell below the target corresponding to loss of carotid or radial pulses (depending on the phase of the protocol, the most cautious in the shock phase). On entering the in-hospital phase of the model, a more aggressive resuscitation to a normotensive target was adopted using PRBCs:FFP (1:1 ratio), representing current best clinical practice. Comparison of these prehospital treatment groups therefore allowed an evaluation of the impact of prehospital blood products in the context of a severely injured casualty given the best treatment immediately on arrival in hospital.

The resuscitation was conducted to defined, clinically relevant, hypotensive pressure targets in the prehospital phases. This was designed to limit the volume of fluid given to the minimum required to sustain life. In the model development phase, failure to provide this fluid was not compatible with survival, emphasizing the severity of the injury/shock model presented here. In the control group (group 1), saline was used exclusively in the prehospital phase because this is still in widespread clinical use. This led to a degree of hemodilution as seen by the fall in hematocrit. However, this degree of hemodilution is a valid part of the overall model because it represents the dilution inherent in prehospital resuscitation using crystalloids (the old/current standard of care). To place the hemodilution in the present study into the context of other models of injury, the volume of 0.9% saline used in group 1 in this study represents approximately 0.68 of the remaining blood volume at the end of the hemorrhage. By contrast, an established model published by Cho et al. (42) administered a volume of saline corresponding to approximately 4.5 times the end hemorrhage blood volume. It is therefore likely that although the coagulopathy seen in the present study

may have an element of dilution, it is not an overwhelming degree of dilution.

It is therefore very unlikely that all of the coagulopathy seen in the present study was due to hemodilution. However, it is important to acknowledge that a dilutional element is probably also present, but the volume of fluid given was kept to a minimum compatible with survival by using clinically relevant resuscitation targets. The results of this study are therefore relevant to a situation where a severely injured casualty demands a limited degree of resuscitation to sustain life during evacuation to a hospital and is therefore relevant for military battlefield casualties (19) and some civilian trauma victims where fluid is given in the evacuation phase (10).

In the present study, we utilized a controlled hemorrhage rather than uncontrolled blood loss, which is important to acknowledge. The results of the present study are therefore most relevant for a casualty in whom the majority of the hemorrhage has been controlled at an early stage either by the patient's own "first clot" or by the effective deployment of the <C>ABC paradigm which targets uncontrolled bleeding from major vessels (43). However, any small vessel bleeding from the extensive tissue contusion that represented the tissue injury element was not controlled externally. Because this represents a large volume of tissue (surface area of damaged vasculature), it may represent a greater potential sink for hemostatic factors than, for example, the uncompressed grade IV liver injury we used in the past as a model to assess potential rebleeding in new resuscitation strategies (44). It is impossible to quantify the impact of this tissue injury on the overall coagulopathy and response to treatment in the present study. By contrast to our model, Sondeen et al. (26) have modeled an uncontrolled truncal hemorrhage by incorporation of a splenic injury. In further contrast to our model, the postinjury resuscitation essentially represents a prolonged (5 h) prehospital phase with limited fluid resuscitation and consequently significant mortality. Both models are valid representations of different scenarios. The results of the two studies are in broad agreement. Use of blood products, even when the ratio of PRBCss to plasma is high, obviated measured clotting abnormalities compared with resuscitation with a starch solution (Hextend) and also reduced blood loss compared with both starch and crystalloid (Ringer's lactate) solutions. It is of interest in the latter study (26) that use of crystalloid was not associated with clotting abnormalities when assessed with TEG and only transiently (one early timepoint) associated with increased PT. The authors (26) suggest that this may be due in part to greater hemodilution and/or direct interference with clotting mechanisms induced by the starch solution.

Thromboelastography was chosen as the primary method of assessing clotting in this study because it is a whole-blood test and is viewed as being superior to "conventional tests" (PT or aPTT) in studies in pigs (45). Reassuringly, however, an aPTT gave a similar overall pattern (although not statistically significant) to that seen with TEG. The initial coagulopathy was also seen when PT was used, although the reversal of coagulopathy seen in the in-hospital phase with both TEG and aPTT was not seen with PT. We have no explanation for this difference between tests, although it has been suggested that aPTT (rather than PT) is becoming increasingly recognized as the better

assessment in trauma coagulopathy (T. Wolley, personal communication oral, April 2015). The present study has a number of limitations. It is an animal-based study, and differences as well as similarities to the human response need to be acknowledged. The hemodynamic response to trauma is broadly similar between humans and pigs, and the splenectomy performed in our study removes the capacity to autotransfuse splenic blood that can occur in pigs but not humans; hence, the pig is a widely accepted model of human trauma. Although there are similarities in clotting between pigs and humans, there are also important differences, e.g., uninjured pigs are hypercoagulable compared with humans. These differences are likely to be of importance when assessing some of the specific component therapies, but they do not obviate the general conclusions regarding patterns of changes described in this report. Caution must always be exercised when comparing between species; patterns of response rather than absolute numerical values should be compared. It is notable in the present study that the coagulopathy was characterized on the basis of clot initiation and dynamics, but no change was seen in clot strength despite a marked coagulopathy developing based on the other parameters. The solution to these general limitations of animal-based studies is to triangulate results between species and models and always make comparisons to humans whenever data are available.

In conclusion, this study provides evidence that use of prehospital blood products confers advantage compared with the old standard of care (limited crystalloid administration) and supports a treatment strategy that was started pragmatically by clinicians (MERT) in Afghanistan. If FFP is not available, then PRBCs alone has a clear benefit over saline. Further studies addressing longer prehospital timelines may be needed for future military operations in austere environments. The use of components such as fibrinogen and other products that do not require refrigeration needs to be examined to reduce the logistical burden associated with prehospital deployment of these important products.

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