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PLASMA EXPANDER AND BLOOD STORAGE EFFECTS ON CAPILLARY PERFUSION IN TRANSFUSION FOLLOWING HEMORRHAGE

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Abstract

BACKGROUND—Treating hemorrhage with blood transfusions in subjects previously hemodiluted with different colloidal plasma expanders, using fresh autologous blood or 2-weeks stored blood, allows identifying the interaction between type of plasma expander and differences in blood storage.

STUDY DESIGN AND METHODS—Studies used the hamster window chamber model. Fresh autologous plasma, 130 kDa starch based plasma expander (HES), or 4% polyethylene glycol conjugated albumin (PEG-Alb) were used for 20% of blood volume hemodilution. Hemodilution was followed by a 55% by blood volume 40 min hemorrhagic shock period, treated with transfusion of fresh or 2-weeks stored blood. Outcome was evaluated one hour post blood transfusion in terms of microvascular and systemic parameters.

RESULTS—Results were principally dependent on the type of colloidal solution used during hemodilution, 4% PEG-Alb yielding the best microvascular recovery evaluated in terms of the functional capillary density. This result was consistent whether fresh blood or stored blood was used in treating the subsequent shock period. Fresh blood results were significantly better in systemic and microvascular terms relative to stored blood. HES and fresh plasma hemodilution yielded less favorable results, a difference that was enhanced when fresh vs. stored blood were compared in their efficacy of correcting the subsequent hemorrhage.

CONCLUSION—The type of plasma expander used for hemodilution influences the short term outcome of subsequent volume resuscitation using blood transfusion; 4% PEG-Alb providing the most favorable outcome by comparison to HES or fresh plasma.

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Keywords

Transfusion; hemodilution; stored blood; plasma expanders; hemorrhage; microcirculation

INTRODUCTION

Treatment of blood loss usually proceeds in sequence where the initial reduction of blood volume is corrected using plasma expanders. Blood is used when the blood loss continues and extends beyond the so called transfusion trigger. Outcome in this process is determined by the restoration of normal microvascular function characterized by the extent to which functional capillary density (FCD, the number of capillaries per unit area of tissue observed with passage of red blood cells [RBCs]) returns to normal.¹

Normal FCD results from the adequate transmission of blood pressure to the periphery, limiting vasoconstriction, and the absence of capillary obstructions due to capillary collapse and abnormal blood cells. Moderate levels of colloidal plasma expansion and hemodilution up to about 50% exchange have no effects on FCD.² However if bleeding follows hemodilution the condition of the organism is significantly affected by the extent of hemodilution and volume loss,^{3–6} a situation described by Van der Linden and Vincent as “tolerance of hemodiluted animals to hemorrhage”.⁵ This is an unusual clinical scenario; however, it can serve as an extreme model for the situation where hemorrhage is treated with plasma expanders and eventually with a blood transfusion. It should also evidence possible interactions between the type of colloid and blood used when these fluids are administered in sequence, which is a likely scenario.

Crystalloid and colloidal based plasma expanders are used in the initial phase of blood volume restoration. New hypotheses are emerging on the relative efficacy of colloid based plasma expanders with significantly different viscosities and colloidal osmotic pressures (COP).^{7–9} The following materials are representative of the spectrum of these properties: hydroxyethyl starch (HES) is a commonly used colloid;^{10–12} polyethylene glycol conjugated albumin (PEG-Alb)¹³ is a new material that consistently yields improved microvascular outcomes in experimental resuscitation scenarios;^{14–19} and human serum albumin (HSA), used for plasma expansion in patients.²⁰ Presently, we used hamster plasma 1 to compare PEG-Alb with albumin, given that plasma is approximately 70% albumin. Hamster plasma is a convenient source of hamster albumin, avoiding potential immunological effects of colloids from different species. PEG-Alb used in this study is generated by Extension Arm Facilitated (EAF) pegylation, a new approach to protein pegylation. EAF pegylation engineers a zone of extension arms, nearly 1 nm in thickness, between the outer PEG-shell and the protein core²¹. In this context, pegylation is proposed to render the albumin protein immune-invisible since the intermediary zone of extension arms functions to maximize shielding of the protein core from macro-environmental effects.

An additional component of this present model is the condition of the blood used in the resuscitation process, since storage is known to affect microvascular perfusion. This poses the question whether the nature of the initial plasma expansion is synergistic with the effects due to blood conditions.

The objective of this study is that of uncovering the interaction between the plasma expander used for the initial treatment of blood losses and the subsequent blood transfusion necessary if bleeding continues. In this study we test the hypothesis that the outcome of resuscitation with a blood transfusion in a hemodilution subject is in part dependent on the colloid used prior to blood transfusion, and we examine whether the nature of the colloid can overcome some of the effects due to blood storage. Results could be used to interpret or guide therapy in treating severe bleeding following preoperative hemodilution.¹⁸ In this study we use the hamster window skinfold chamber model to compare the microvascular effects of hemodilution with fresh plasma, HES, or 4% PEG-Alb followed by hemorrhagic shock treated by restoration of oxygen carrying capacity with fresh blood or 2-weeks stored blood.

MATERIALS AND METHODS

Animal preparation

Studies were performed in golden Syrian male hamsters (Charles River Laboratories, Boston, MA), weight range of 56–71 g. The Guide for the Care and Use of Laboratory Animals (US National Research Council, 2010) was followed for animal handling. Experiments were approved by the University of California, San Diego Institutional Animal Care and Use Committee. The window chamber model was utilized for microvascular studies in unanesthetized hamsters. Chamber implantation and vascular catheterization surgeries were performed under general anesthesia, pentobarbital 50 mg/kg or ketamine/xylazine cocktail 200/10 mg/kg i.p. injections, as previously described.^{22,23} Following chamber implantation, animals recovered for a minimum two day period prior to catheterization. Animals were anesthetized for carotid artery and jugular vein catheter implantation (polyethylene-50), following microscopic chamber assessment in order to rule out edema, bleeding, or signs of infection. Animals were entered into the study one to two days following catheterization surgery.

Systemic parameters

Mean arterial blood pressure (MAP) and heart rate (HR) were continuously monitored using a Biopac system (MP 150; Biopac Systems, Inc., Santa Barbara, CA) excluding blood sampling, hemodilution, and hemorrhage periods, when the arterial catheter was in use. Systemic hematocrit (Hct) was measured from arterial blood collected using heparinized microcapillary tubes, following centrifugation. Baseline systemic parameter requirements for animal inclusion in the study were: MAP > 80 mmHg, HR > 320 beats/min, and systemic Hct > 40%.

Functional Capillary Density

FCD was determined in 10 stepwise vertically successive microscopic fields using 20X magnification. Capillaries were considered functional having at least one transiting RBC, during a 30 sec observation period. FCD (cm^{-1}) is the total length of RBC-perfused capillaries divided by the surface area of tissue in which they are observed.²³ Initial tissue fields were chosen by a distinguishing anatomical feature to allow quick and accurate recognition during repeated measurements.

Plasma expander fluids and experimental groups

Experiments were carried out in 3 groups of hamsters (n=12 each group), each group corresponding to a plasma expander used for hemodilution (PEG-Alb, HES, or plasma). Each plasma expander group was further divided into two groups (n= 6 in each subdivided group), corresponding to the type of blood used during resuscitation following hemorrhage: one group received fresh autologous blood and the other group was treated with stored allogeneic whole blood. Animals were randomized into both group divisions.

Albumin pegylation has been previously described.^{18,24} Fresh plasma was obtained the same day of experiments from a donor hamster. HES is a 130 kDa starch molecule with a low degree of molar substitution (Voluven; Fresenius-Kabi, Graz, Austria).²⁵ EAF pegylation of albumin was carried out in the laboratory of Prof. Acharya at Albert Einstein School of Medicine, The Bronx, NY as previously described.²¹ Briefly, lyophilized preparations of albumin from Sigma Aldrich were subjected to EAF pegylation at a temperature of 4 °C and a protein concentration of 0.5 mM, using 10 mM maleimidophenyl PEG 5K (custom synthesized), in the presence of 5 mM 2-IT. The hexaPEGylated albumin thus generated was then purified through tangential flow filtration and concentrated to a 4 gm % solution and stored at -80 °C. The physical properties of the plasma expanders used are given in Table 1.

Collection and storage of whole blood and plasma

Fresh autologous blood collection—Blood withdrawn from the carotid artery catheter was collected in 5 ml syringes containing citrate phosphate dextrose adenine 1 anticoagulant solution (Fenwal, Inc., Lake Zurich, IL, CPDA-1). The amount of CPDA-1 solution used was 0.14× total blood volume (BV) withdrawn.

Stored blood—Blood withdrawn from the carotid artery catheter of a donor hamster was collected into a 5 ml syringe containing CPDA-1 (0.14× total BV = 4.56 ml). Blood was then transferred under sterile conditions to a sterile preservative-free vacutainer tube and immediately placed in 4°C storage for a 14–15 day period.

Fresh plasma collection—Fresh plasma was obtained the same day as used from a donor hamster and collected in citrate. Donor blood was centrifuged, and plasma was separated from RBCs.

Experimental setup and acute isovolemic exchange transfusion hemorrhagic-shock and resuscitation protocol

The experimental procedure was the same for all 6 animal groups. Unanesthetized animals were placed in a restraining tube, stabilized by securing the tube and the chamber to a plexiglass stage. The plexiglass stage holding the animal was placed on an intravital microscope (BX51WI; Olympus, New Hyde Park, NY) equipped with a 20X objective (LUMPFL-WIR, numerical aperture 0.5; Olympus). Animals were given 15–30 min to adjust to the tube environment before baseline measurements were taken (MAP, HR, FCD, and Hct).

Following baseline measurements, an acute anemic state was induced by a 20% of BV (estimated as 7% of individual body weight) hemodilution, through an isovolemic exchange transfusion with either 4% PEG-Alb, HES, or fresh plasma in citrate using a dual syringe pump (model 33 syringe pump; Harvard Apparatus; Holliston, MA). The plasma expander was infused into the jugular vein catheter and blood was withdrawn from the carotid artery catheter at a rate of 100 μ l/min. Animals were then given a 30 min stabilization period, during which FCD was measured. Systemic parameters were measured subsequent to the 30 min period, prior to the start of the two-step hemorrhage procedure. Following the stabilization period animals were subjected to a two-step hemorrhage procedure. The volume of each hemorrhage step was a calculated percentage of the animal's BV. Step one and two were 40% and 15% of BV, respectively, each carried out in a 5 min period, with a 20 min waiting period given between the first and second step of hemorrhage. Resuscitation was initiated after 10 min with a 62.7% of BV blood transfusion using either fresh autologous or stored whole blood. Resuscitation volume (62.7%) included 55% (of BV) of hemorrhaged whole blood, the balance being anticoagulant.

Fresh autologous blood was maintained at room temperature until resuscitation. Stored blood was removed from storage and allowed to warm to room temperature 30 min prior to resuscitation. Results were evaluated at 60 min after transfusion at which time the experiment was terminated. FCD and systemic parameters were assessed as shown in the experimental time table (Figure 1).

Assessment of RBC quality and storage lesions

The fraction of transfused RBCs retained after 24 hr was used to evaluate their quality, in addition to being an indicator of storage lesions. We determined this by examining 24 hr post transfusion survival of hamster RBCs using an ex vivo fluorescent labeling technique. Assessment was completed with fresh RBCs and with RBCs following 2 weeks of storage. RBCs were obtained fresh from donor hamsters and labeled immediately after being drawn with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Frontier Scientific) by a technique that has been slightly modified from that previously described in detail.²⁶ Briefly, after blood was removed from the donor hamster, RBCs were isolated by successive washing and centrifugation. One hundred μ l of RBCs were incubated for 30 min in a mixture of 10 ml Hank's balance salt solution and 100 μ l of 0.5 mg/ml DiI in DMSO. After washing and centrifugation, DiI-labeled RBCs (DiI-RBCs) were re-suspended in human serum albumin (5%, Bayer) in a 2 to 1 ratio. This was then infused into the animal via the venous catheter at a rate of 150–200 μ l/min. Labeled and unlabeled cells were counted at 5 min and 24 hr after infusion of the RBCs using a hemocytometer (Fisher Scientific). Results show that 24 hr after infusion there was a loss of $24.2 \pm 10.2\%$ of fresh cells and $35.7 \pm 13.2\%$ of 2 weeks stored RBCs relative to the count at 5 min.

Statistical analysis

Results are presented as mean $\pm$ standard deviation, unless otherwise noted. Data are presented as absolute values and/or as values relative to baseline values. All measurements were compared with baseline levels obtained prior to the start of experimental procedures. The same capillary fields were followed in order to enable direct comparisons to their

baseline levels, allowing for more robust statistics for small sample populations. For repeated measurements, within groups, time-related changes were assessed by ANOVA for nonparametric measurements (Kruskal-Wallis), and when appropriate post hoc analyses performed with the Dunn's multiple comparison test. Changes between groups were assessed using the Mann-Whitney test. All statistics were calculated with computer software (Prism 4.0, GraphPad, San Diego, CA). Changes were considered statistically significant if $p < 0.05$.

RESULTS

Animals studied throughout the experimental procedures completed the hemodilution phase with a MAP change no greater than 10 mmHg, indicating a successful exchange transfusion.

Hematocrit

Hct for all animal groups was decreased following hemodilution as expected and then again following the two-step hemorrhage procedure, with significant decreases resulting in all groups compared to both baseline and hemodilution values (Figure 2). Hcts were restored to post hemodilution levels, not being significantly different, after the 60 min recovery period with no significant differences between groups resuscitated with fresh autologous blood. Animals resuscitated using stored blood increased Hct following resuscitation, returning to post hemodilution values, however, remaining significantly decreased compared to baseline levels. Transfusion of stored blood caused a significant increase in Hct 60 min post infusion for all plasma expander pre-treatments, relative to the fresh blood transfused animals ($p < 0.05$, Figure 2).

Mean arterial pressure

Hemodilution reduced MAP relative to baseline; however, the change was not significant. MAP decreased significantly after hemorrhage, relative to baseline and hemodilution measurements, this downward trend was mirrored by the fresh autologous and allogeneic stored blood animal groups for each of the three individual plasma expanders ($p < 0.05$, Figure 3). Fresh and stored blood MAP showed similar trends, 60 min post resuscitation. MAP increased for all groups, the effect being greater for the stored blood group ($p < 0.05$, Figure 3), a trend similar to that found with restored Hct levels.

Heart rate

Hemodilution and hemorrhage decreased HR, which was returned to near normal values by blood transfusion to an extent depending on the plasma expander used during hemodilution. The 4% PEG-Alb groups had significantly diminished HRs due to hemorrhage compared to baseline ($p < 0.05$, Figure 4). The HES animal groups only showed a significant HR variation in the fresh autologous blood resuscitated group. There were no significant differences between the HRs of HES fresh autologous and stored blood resuscitated groups. Similarly, HRs of plasma hemodiluted animals resuscitated with fresh autologous blood attained a significant difference between the post recovery period and hemorrhage values, while HRs of plasma animals resuscitated with stored blood were significantly different following hemorrhage compared with baseline and hemodilution ($p < 0.05$). Differences

between plasma fresh autologous and stored blood resuscitated animal group HRs were not significant (Figure 4).

Blood gases and base excess

Peg-Alb, HES, and plasma animal groups' respiratory stability was maintained during the experimental protocol preceding the 15% hemorrhage, which caused hyperventilation and the rise in PO₂. The difference in PO₂ levels between fresh and stored blood resuscitation groups for all plasma expanders increased by comparison to baseline levels, following hemorrhage. PO₂ levels remained elevated 60 min post resuscitation relative to baseline. However, only the PO₂ levels of the PEG-Alb and HES animal groups remained significantly increased. The plasma animal groups returned to baseline levels (Figure 5).

Additionally, the Peg-Alb, HES, and plasma groups showed a dramatic fall in base excess, following hemorrhage, corresponding to the animal's hyperventilated state, compared to baseline. Resuscitation with both fresh and stored blood restored base excess values for all groups, with the exception of HES fresh blood, 60 min post resuscitation (Figure 6).

Although the group receiving Peg-Alb hyperventilated during hemodilution the acid-base response to hemorrhage was more stable. Peg-Alb animals maintained a base excess closest to baseline levels by comparison to the other two plasma expander groups, although having similar if not lower hematocrits (Figures 2 and 6).

Functional Capillary Density

Hemodilution did not significantly affect FCD, however, a slight decrease from baseline was observed in all animal groups. This trend continued during the hemorrhage phase of the protocol, causing a significant decrease in FCD that was most pronounced for HES and fresh plasma (Figure 7). Resuscitation with blood restored FCD to near 100% of baseline levels in the PEG-Alb/fresh blood group. Recovery of FCD was progressively less in the HES and fresh plasma groups, following fresh blood transfusions. The same trend of recovery of FCD was found for stored RBCs, however, overall recovery levels were lower (Figure 8). Four percent PEG-Alb maintained FCD during hemorrhage and produced the highest FCD 60 min after resuscitation ($p < 0.05$, Figure 8A).

DISCUSSION

The principal finding of this study is that the type of colloid used in the initial hemodilution of blood volume restitution affects the outcome FCD when blood is transfused to correct a subsequent hemorrhage. Hemodilution with PEG-Alb and transfusion with fresh blood yields full recovery of FCD while the fresh plasma/stored blood combination results in 50% recovery. Fresh blood yields a higher level of restoration of FCD than stored blood for any given plasma expander used in the prior hemodilution and the type of plasma expander used makes a difference on the final FCD attained.

The same trend in the effects of plasma expanders were observed for both fresh and 2-weeks stored blood. However, fresh blood yielded a consistently higher FCD upon transfusion by comparison to stored blood, an effect consistent with previous findings on the effects of

storage on RBC properties.²⁷ Similar results were found in the study of Gonzalez et al.²⁸ who subjected rats to a 10% blood loss that were subsequently treated with fresh blood and 1-week and 2-weeks old stored blood. In this study the principal effect is reported to be significant 4 hr after transfusion, consisting in an approximately 8% decrease in FCD, with no difference between one and 2-weeks storage. This rather small effect is probably a consequence of the significantly smaller transfusion performed in their study, 10% of blood volume vs. 55% in our study.

Base excess was the same in all groups after blood transfusion. However the recovery of base excess, i.e., the change between hemorrhage (H15%) and after transfusion (R60) was greater for plasma and HES hemodilution. This in part reflects the lesser decrease of base excess with PEG-Alb hemodilution due to the greater FCD during hemorrhage (Figure 8).

Differences due to the colloid used for hemodilution were not apparent from the measurement of MAP and HR. The differences in systemic outcome of transfusing fresh or stored blood are evident by blood pressure being significantly elevated for the latter, by comparison with fresh blood. This is probably due to vasoconstriction induced by hemoglobin being liberated from RBCs during storage and from hemolysis due to increased cell fragility of the cell in the stored blood, as evidenced by the presence of plasma hemoglobin in the range of 0.1–1.7 mg/dl.

The type of plasma expander used in the initial treatment of blood losses, simulated in this study by performing a 20% hemodilution, caused effects on FCD that were practically the same for both types of blood transfusion as shown in Tables 3 and 4. However, there were significant difference in microvascular conditions between the best (4% PEG-Alb and 1 fresh whole blood) and worst (Fresh plasma and stored whole blood) scenarios. This interaction between blood storage times and pre-existing blood composition appear to be primarily related to how the initial plasma expansion affects microvascular function, prior to subsequent blood losses and transfusion, since the trend is already present during the shock period when the plasma expander is on board. In this context FCD is significantly higher during the shock period in animals hemodiluted with 4% PEG-Alb than when using either HES or plasma.

As in our previous work, and that from other investigators, transfusion with fresh RBCs leads to a better outcome than using stored blood. One of the earliest investigations of this effect in conjunction with resuscitation in hemorrhagic shock was reported by Collins and Stechenberg,²⁹ who used a model of severely stressed rats. The model consisted in exchange transfusing 90% of the rat's blood volume with either fresh blood or 14–20 day stored blood at different hematocrits (17, 25, and 34%), inducing an initial state of anemia, causing hemorrhage (~50% of blood volume) and resuscitating with the same blood originally exchanged. This study showed that a difference in survival between fresh and stored blood was only evident for the anemic subjects and attributed this difference to the reduction of oxygen supply to the tissue, from the reduction in Hct and the increased oxygen affinity due to the depletion of 2, 3-DPG in the stored blood.

The present study and related results from microvascular studies suggest that the results of the Collins and Stechenberg experiment and similar studies are more specifically related to the effects on capillary flow *per se* rather than oxygen supply. Kerger et al.³⁰ showed that extended untreated hemorrhage survival correlated with the maintenance of a threshold FCD, independently of tissue oxygen, substantiating previous reports of the association between survival and improved perfusion in skeletal muscle.³¹ Cebrales et al.³² reported that increased oxygen affinity in anemia improves microvascular function and Sakai et al.³³ showed that high oxygen affinity hemoglobin improves tissue oxygenation in anemia. Taken as a whole, these and other results suggest that the organism and tissue survive a considerable 1 reduction of Hct provided that FCD is maintained. In this scenario, however, the introduction of stored RBCs has the potential of inducing negative effects because of the reduction of FCD, probably due to their reduced flexibility³⁴ causing capillary obstruction. The associated hemolysis, liberating hemoglobin in plasma, may be an additional factor since vasoconstriction can reduce capillary pressure.³⁵

In previous studies we determined the correlation between microvascular hemodynamics responses in the tissue of the window skinfold chamber and major organs;⁷ however, subcutaneous/skeletal muscle tissue cannot be compared to internal tissues/organs such as the kidney, heart, or viscera. In this context our model is a compromise between observations of microhemodynamic effects in an unexposed intact tissue, without anesthesia, and studying responses in the major organs where microhemodynamic measurements cannot be made.

The mechanisms behind the effects found using 4% PEG-Alb as a plasma expander are still under study. The molecule was originally developed by Abuchowski et al.,³⁶ but was not extensively investigated until recently. PEG-Alb is a comparatively large molecule that immobilizes a significant amount of water on its surface. It has a relatively high COP (42 mmHg at 4% concentration), which limits its concentration in the circulation. As a consequence its potential viscogenic effect is reduced; however, its physiological effect remains similar to that of high viscosity plasma expanders,³⁷ which consists in a significant improvement of microvascular function in extreme anemic conditions. Four percent PEG-Alb is not particularly viscous, since its effective viscosity in plasma is decreased by dilution. The beneficial effects of 4% PEG-Alb appear to be due to a combination of factors, including hemodilution, which reduced overall blood viscosity and increases blood flow, a modest increase in plasma viscosity, and increased NO bioavailability³⁸ by mechanotransduction in the endothelium due to increased flow. There is clearly a need for further investigations.

We previously showed that microvascular and tissue dysfunction during shock treatment with HES occurs 15 minutes after the initiation of treatment.¹⁵ In the present study, focusing on events occurring at the initial stages of shock resuscitation, we show that this dysfunction is only partially corrected by the restoration of oxygen carrying capacity because the critical restoration of FDC is limited and dependent on blood storage and previous events. Notably, secondary decompensation, in terms of decreased microvascular flow observed during the first 20 to 40 min of post resuscitation shock treatment in the microcirculation of the skeletal muscle, the ileum, and the kidney^{39,40} was not observed.

The present model is not a usual clinical scenario, since it is unlikely that a hemodiluted patient would undergo hemorrhagic shock. However it does answer the question, if a hemodiluted patient were to go into shock, what plasma expander used for hemodilution results in the better outcome?

We sought to determine the interaction between plasma expanders, used for the initial treatment of blood loss, and the subsequent blood transfusions when blood loss continues. Results of our study may be used to interpret or guide therapeutic procedures during severe bleeding and subsequent preoperative hemodilution.¹⁸ Additionally, these results provide a broader basis for understanding the tolerance to hemorrhage following hemodilution, previously investigated in terms of the level of volume restitution and the effects of oxygen delivery capacity associated with the level of hemodilution.^{3–6,41}

In conclusion, the use of stored blood consistently yields decreased microvascular function by comparison with fresh blood, an effect reflected by many studies comparing the effects of blood storage.⁴² Outcome in terms of microvascular function when a blood transfusion is made following initial blood volume restoration with plasma expanders is shown to depend on the type of plasma expander, results being optimal for 4% PEG-Alb, and progressively worst for HES and homologous plasma, a result that appears to be due to effects inherent to the plasma expander on the microcirculation. In other words, the microvascular outcome following a blood transfusion is dependent on how the organism and the microcirculation react to the plasma expander used prior to a blood transfusion. In addition, the microcirculation responds differentially depending on the extent of blood storage. As a corollary it appears possible to diminish the microvascular effects due to blood storage by improving pre-transfusion microvascular function.

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Figure 1.

The experimental protocol. Time course of the acute isovolemic exchange transfusion/hemodilution with 4% PEG-Albumin, Hydroxyethyl starch, or plasma, the two-step hemorrhage procedure, and resuscitation with either fresh autologous or stored whole blood.

Figure 2.

Changes in hematocrit due to hemodilution. Baseline hematocrit (n=36): $48.0 \pm 2.2\%$. Analysis within treatment groups: $p < 0.05$ *vs. baseline. Analysis between treatments at the same time point: $p < 0.05$ ^a vs. PEG-Alb Fresh blood; ^b vs. HES Fresh blood; ^c vs. HES Stored blood; ^d vs. Plasma Fresh blood. 4% PEG-Albumin (PEG); Hydroxyethyl starch (HES); Plasma (PLS).

Figure 3.

Effect of hemodilution, hemorrhage and resuscitation on blood pressure. Baseline MAP (n=36): 112.8 ± 9.6 mmHg. Analysis within treatment groups: $p < 0.05$ *vs. baseline; † vs. hemodilution; ‡ vs. 15% H. Analysis between treatments at the same time point: $p < 0.05$ ^a vs. PEG-Alb Fresh blood. 4% PEG-Albumin (PEG); Hydroxyethyl starch (HES); Plasma (PLS).

Figure 4.

Effect of hemodilution, hemorrhage and resuscitation on heart rate. Baseline HR (n=36): 464.8 ± 49.1 beats/min. Analysis within treatment groups: $p < 0.05$ *vs. baseline; † vs. hemodilution; ‡ vs. 15% H. Analysis between treatments at the same time point: $p < 0.05$ ^a vs. PEG-Alb Fresh blood; ^b vs. HES Fresh Blood. 4% PEG-Albumin (PEG); Hydroxyethyl starch (HES); Plasma (PLS).

Figure 5. Effect of hemorrhage and resuscitation on arterial PO_2 . Baseline (n=36): PO_2 56.5 ± 5.8 mmHg. Analysis within treatment groups: $p < 0.05$ *vs. baseline; ‡ vs. 15% H. Analysis between treatments at the same time point: $p < 0.05$ ^a vs. PEG-Alb Fresh blood. Hemorrhage (H) and 60 min post resuscitation (R60). 4% PEG-Albumin (PEG); Hydroxyethyl starch (HES); Plasma (PLS).

Figure 6.

Effect of hemorrhage and resuscitation on arterial base excess. Baseline (n=36): Base excess 5.8 ± 2.0 . Analysis within treatment groups: $p < 0.05$ *vs. baseline; ‡ vs. 15% H. Analysis between treatments at the same time point: $p < 0.05$ ^a vs. PEG-Alb Fresh blood. Hemorrhage (H) and 60 min post resuscitation (R60). 4% PEG-Albumin (PEG); Hydroxyethyl starch (HES); Plasma (PLS).

Figure 7.

Fresh autologous blood versus stored blood. Analysis within the same treatment group 4% PEG-Albumin (A), HES (B), or fresh plasma (C): $P < 0.05$ relative to BL (*), Post Hemodilution (†), Post15% Hemorrhage (‡). Analysis between treatments at the same time point: $P < 0.05$ (§). 4% PEG-Albumin (PEG-Alb); Hydroxyethyl starch (HES); Fresh autologous blood (FB); Stored blood (SB).

Table 1

Properties of PEG-Albumin, HES, and plasma.

	PEG-Albumin	HES	Plasma
Source	Synthetic/Human	Synthetic	Hamster
Concentration (%)	4	6	–
COP mmHg	42	42	16
Viscosity (cP)	2.2	2.1	1.2
Average molecular weight (kDa)	96	130	–
Suspending fluid	Phosphate buffer	Saline	–
Degree of substitution	–	0.40	–

Hydroxyethyl starch (HES); Colloid osmotic pressure (COP).