Comparison of Viscoelasticity after Infusion of Blood Products in a New Splenic Injury Swine Hemorrhage Model

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Introduction

We are interested in developing a resuscitation solution which not only restores plasma volume but acts as a hemostatic agent to promote clotting and reduce blood loss, particularly in truncal non-compressible bleeding. We have therefore developed a combined controlled (24 ml/kg) and uncontrolled hemorrhage model in the pig using a spleen injury (see Sondeen et al poster). We are comparing the standard of care resuscitation solutions, lactated Ringer's solution (LR) and Hextend, to various ratios of blood products: fresh frozen plasma (FFP), fresh non-autologous whole blood (FWB) which contains normal levels of platelets, and a 1:1 and a 1:4 ratio of FFP and packed RBC, which have been filtered to remove platelets. We are measuring the amount of blood loss after hypotensive (no higher than 65 ± mmHg mean arterial pressure) resuscitation with the different fluids at 15 (colloids) or 45 (LR) ml/kg. This low volume of resuscitation fluid models that which a medic might be able to carry in a small golden hour box (~4 units FFP).

Several groups (1,2) have demonstrated that resuscitation with a higher viscosity fluid leads to greater functional capillary density in the microcirculation and improved oxygen delivery to the tissues. We were concerned that if flow in the microcirculation is maintained in the presence of an injury and uncontrolled hemorrhage, bleeding may be increased because more vessels would be open. The resuscitation fluids that we are testing in our study have a range of viscosities: LR viscocity with a Poise 0.009 is similar to water, Hextend with a Poise 0.040 is similar to blood, plasma is 0.014 Poise, and blood at low shear rate is approximately 0.040 Poise. Another change in blood rheology that has been shown to occur following hemorrhagic shock is a reduction in the RBC deformability, which may lead to organ dysfunction (3).

Our goal is to measure the changes in the viscosity and elasticity of the pig's blood and the red cell deformability following resuscitation with various fluids, and to see if there is an effect on blood loss in an uncontrolled hemorrhage model in swine. We are using a BioProfiler Blood Viscoelastography Analyzer (Vilastic Scientific, Austin, TX) to measure viscosity, elasticity and RBC deformability. Viscoelasticity of swine blood has not been studied, particularly after hemorrhage and the use of blood products as resuscitation fluids. Normal hematocrit of human blood is about 40-45, much higher than a normal hematocrit of 30-35 in swine. The Bioprofiler states it can determine viscosity and elasticity in blood with hematocrits ranging from 50% to 17%.

Objectives

To compare the viscosity and elasticity of blood in hemorrhaged, splenic injured and resuscitated swine with blood products or conventional fluids. The blood products include Fresh Frozen Plasma (FFP), Fresh Whole Blood (FWB), and Packed Red Blood Cells (PRBC). Conventional fluids used are Hextend and Lactated Ringers Solution (LR). We will also compare differences in deformability of red blood cells following resuscitation.

Methods

The Bioprofiler uses increasing oscillatory shear rates and shows influences of aggregation, disaggregation, cell orientation and cell deformation on the viscoelasticity of blood. There are 3 significant regions of shear rates that can define blood flow. At low shear rates (Fig 1, 1 – 10/sec), the cells are formed into large aggregates. As the shear rate increases (Fig 2, 10 – 100/sec), the size of the aggregates decreases. At high shear rates (Fig 3, 100 - 1000), increasing shear stress deforms the cells, and if the cells have normal deformability they will form layers within the plasma. Cells with impaired deformability (increase of cell rigidity) form thicker cell layers with thinner plasma layers, thus increasing viscosity and elasticity. Deformability of cells is needed for perfusion into the capillary beds.

Blood with low aggregation tendencies will form smaller than normal aggregates when at low shear rates. As a result (Fig 4), the low shear viscosity and elasticity of blood with reduced aggregation tendencies (red) are below the values for normal blood (blue). At low shear rates, the viscosity and elasticity of blood with elevated aggregation tendencies (green) are above those for normal blood. In the high shear region, aggregation effects no longer dominate and the ability of RBCs to form laminar flow prevails. At high shear stress, cells with a diminishment of deformability will have difficulty forming laminar flow and the tail region of the curve is elevated. Fig 5 shows the idealized effects on viscoelasticity of blood containing cells with low deformability (red) as compared to the viscoelasticity of blood containing cells of normal deformability (blue).

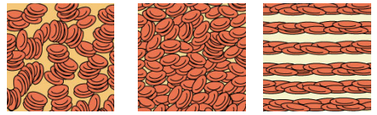
EXPERIMENTAL PROCEDURE:

- 35-45Kg female pigs, were anesthetized with isoflurane.
- Catheters were placed, via cutdowns, in the left femoral artery vein, and left carotid artery and jugular vein. A Foley catheter (14Fr) was placed in the bladder via a cystotomy. A laparotomy was also performed for access to the spleen.
- A Baseline blood sample was taken when the animal stabilized.
- An initial controlled hemorrhage of 24ml/Kg was performed.
- Spleen was then cut through and through along its entire length starting one inch from the hilum. Hemorrhage volume from spleen injury was measured.
- Fifteen minutes after spleen injury, fluid resuscitation was started.
- Blood samples were collected at Baseline, 15 minutes post spleen injury, and 60 minutes post spleen injury.
- The sample size for the Bioprofiler is measured at 1ml of blood taken from a purple top blood tube, collected at each time point, concentrated with 3.6mg EDTA. Viscosity and elasticity were calculated by the Vilastic Software.

Fig 1. Low Shear Rate: In quiescent state aggregates form

Fig 2. Mid Shear Rate: Cells Orient and start to deform

Fig 3. High Shear Rate: Cells form layers with plasma layers



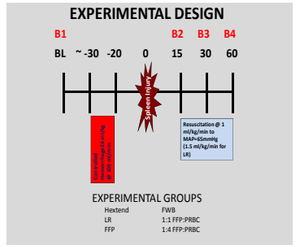


Fig 4. Influence of Red Cell Aggregation

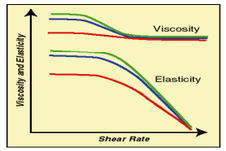
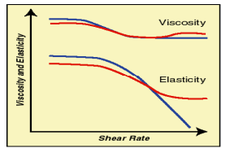
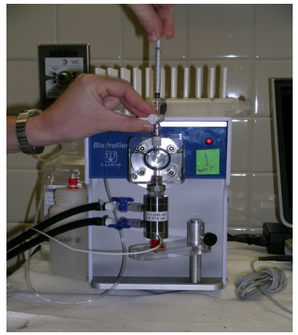


Fig 5. Influence of Red Cell Deformability





Results and Discussion

Viscosity

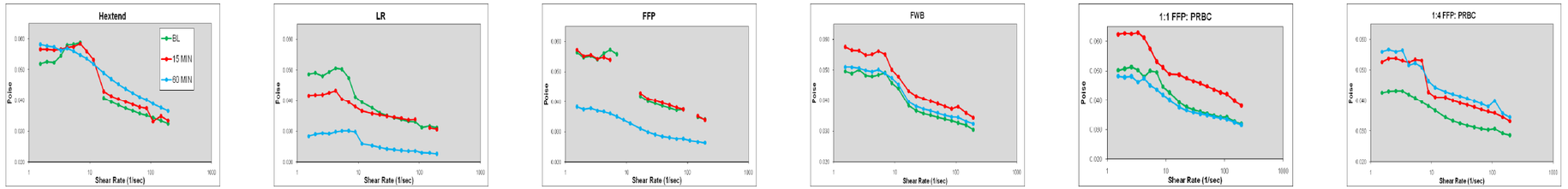


Fig. 6. These figures show the 20-point viscosity profile of whole blood samples. The data from individual animals (n=4 – 6/ group) were normalized by interpolation to the average shear rate, then plotted. Error bars in the graphs are omitted for clarity. The baseline samples (green) for the six groups were relatively similar. The rise in viscosity between shear rates of 1-10/ sec may indicate that some pigs have a tendency increase their aggregation and hence viscosity, prior to disaggregating. The viscosity of the blood following hemorrhage (red) showed quite a lot of variability among the groups, although they had all received similar treatments to this point. The pig has a contractile spleen, and it is possible that some of the variability may be due to some pigs increasing their hematocrit following the splenic injury, although that is not supported by the small, if any, changes in hematocrit (Fig. 8). Resuscitation (blue) with the low-viscosity fluids (LR and FFP) resulted in a reduction of viscosity. Although the hematocrit was reduced to a similar degree in the Hextend group compared to the LR and FFP groups (Fig. 8), the viscosity was not reduced. Resuscitation with FWB and 1:1 FFP:RBC fluids resulted in a return of viscosity to baseline levels. In contrast, resuscitation with the 1:4 FFP:PRBC fluid maintained the viscosity at higher levels, probably due to the higher hematocrit.

Elasticity

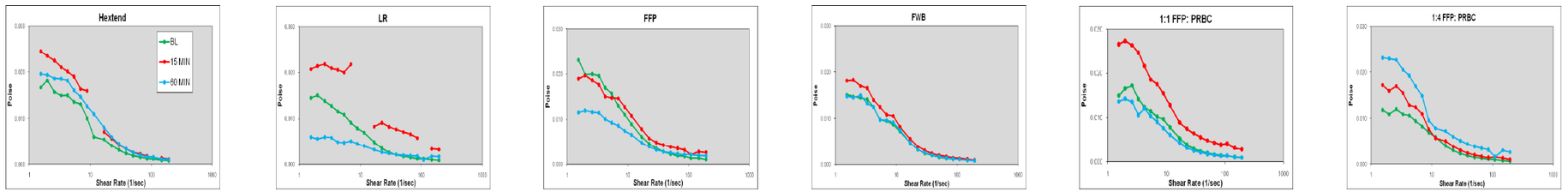


Fig. 7. These figures show the 20-point elasticity profile of whole blood samples. The data from individual animals (n=4 – 6/ group) were normalized by interpolation to the average shear rate, then plotted. Error bars in the graphs are omitted for clarity. The elasticity of the baseline elasticities (green) for the six groups were relatively more variable among the groups than the viscosity (Fig. 6). The elasticity of the blood following hemorrhage (red) generally showed no consistent change. There was not a consistent effect to show reduced deformability at high shear rates (elevated tail region, see Fig. 5), unlike others have claimed (3), but this could be due to different methods of measurement being used. There was a reduction in elasticity at low shear rates in the LR and FFP groups in contrast to the Hextend group. Following FWB and 1:1 FFP:PRBC resuscitation, the elevated elasticity following hemorrhage was returned to baseline. Resuscitation with the 1:4 FFP:PRBC was the only group that showed a trend toward elevated elasticity (reduced deformability) at the high shear rates.

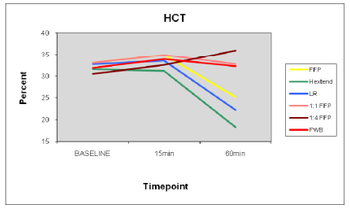


Fig. 8. The hematocrit showed a variable change following hemorrhage, a reduction in the non-PRBC groups, a maintenance in the FWB and 1:1 FFP:PRBC groups, and an increase in the 1:4 FFP:PRBC group.

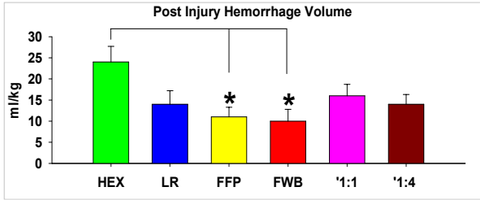


Fig. 9. Hextend resulted in double the post-injury blood loss compared with FFP and FWB. There was a trend for a reduced post-injury blood loss with the other 3 groups as well. This may be consistent with the higher viscosity Hextend keeping more microvessels open resulting in more bleeding.

Summary and Conclusions

- This data is from an interim analysis at n=6 per group. The study is ongoing for a total of n=8 per group.
- Viscosity responses compared to baseline:
  - LR and FFP resulted in a reduction of viscosity
  - Hextend increased viscosity
  - FWB, 1:1 and 1:4 groups showed no change
- Elasticity compared to baseline:
  - LR and FFP showed a reduction at the low shear rates
  - Hextend showed a small change
  - FWB and 1:1 FFP:PRBC elasticity returned to its baseline
  - 1:4 FFP:PRBC decreased in deformability of red cells
- There was more bleeding in the Hextend group compared to the other groups. The elevated viscosity, in the absence of additional clotting factors such as plasma or platelets, may have kept more capillaries open and contributed to increased bleeding.
- In general, the measurements will aid the selection of best resuscitation fluid with the optimum rheology for hemodynamic effects in conditions of uncontrolled hemorrhage.

Acknowledgments

Nancy Henderson, Ph D., Vilastic Scientific.

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