Effect of Conventional Fluids (LR, Hextend) or Blood Products (FWB, FFP, FFP:PRBC ratios of 1:1 and 1:4) on a New Splenic Injury Model in Anesthetized Swine

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Introduction

We are interested in developing an intravenous hemostatic agent that will not only replace plasma volume in hemorrhaged patients, but reduce uncontrolled hemorrhage. Previously, we developed a poly-trauma model (femur fracture, 60% hemorrhage, Grade V liver injury, hypothermia, dilutional coagulopathy) with which we compared blood components (FFP alone, FWB, or 1FFP:1RBC versus LR or Hextend) and found that all of the blood products improved the coagulation status as measured by standard laboratory tests (eg., PT) (1-3). However, there were no changes in uncontrolled blood loss, so there was no evidence that reversing a coagulopathic blood profile resulted in the desired reduction in uncontrolled hemorrhage.

We therefore adapted Dr. Kheirabadi's successful rabbit spleen injury model (4) to the pig. In addition, we wanted to see the effect of limiting the volume on survival. A volume of ~15 ml/kg was chosen, which is equivalent to 4 units of FFP, or 2 FFP + 2 RBC, a volume that a medic might have available in the field using a small golden hour box.

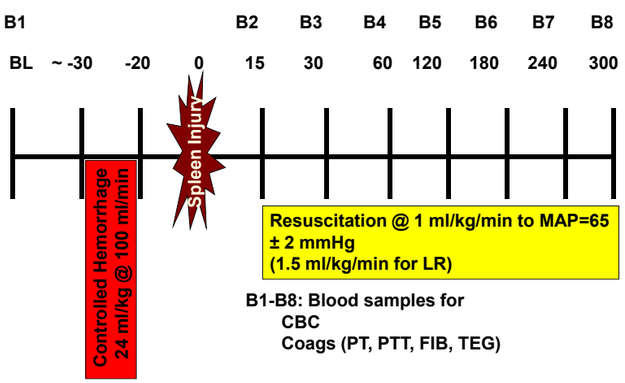
Objective

To develop a combination of severe controlled hemorrhage and uncontrolled splenic injury hemorrhage model in swine to test whether hypotensive resuscitation with blood products is more beneficial than conventional fluids in reducing blood loss.

Methods

- 35-45Kg female pigs (Midwest Research Swine, Gibbon, MN), were anesthetized with isoflurane.
- Catheters for sampling blood and measuring blood pressure were placed, via cutdowns, in the left femoral artery vein and left carotid artery and a Swan-Ganz catheter for measuring continuous cardiac output was placed in the pulmonary artery via the 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 24 ml/Kg was performed at 100 ml/min.
- 20 minutes after completion of the controlled hemorrhage, the spleen was then cut through and through almost along its entire length starting one centimeter from the hilum. Hemorrhage volume from spleen injury was measured continuously by suction into vacuum flasks on a balance connected to a computer.
- Fifteen minutes after spleen injury ,resuscitation fluids were started (15.25 ml/kg for colloids, 45.75 ml/kg for LR). Using a closed loop Labview-based computer system, resuscitation was targeted to a MAP=65 mmHg until the complete volume was given (usually completed by 60 min).
- The animals' hemodynamics and survival were measured for the next 6 h. Blood samples for coagulation tests, hematocrit, and complete blood count were collected hourly.

EXPERIMENTAL DESIGN



Groups (n=6/ group)

1. Hextend: 15.25 ml/kg at 1 ml/kg/min
2. LR: Lactated Ringer's – 45.75 ml/kg (3x colloid volume) at 1.5 ml/kg/min
3. FFP: Fresh frozen plasma [FFP] – 15.25 ml/kg at 1 ml/kg/min
   1FFP:1RBC: 1:1 ratio FFP to filtered (no platelets) packed red blood cells [FFP:RBC] – 15.25 ml/kg at 1 ml/kg/min
4. 1FFP:4RBC: 1:4 ratio of FFP:RBC – 15.25 ml/kg at 1 ml/kg/min
5. FWB: fresh whole blood (contains platelets) -15.25 ml/kg at 1 ml/kg/min

Results and Discussion

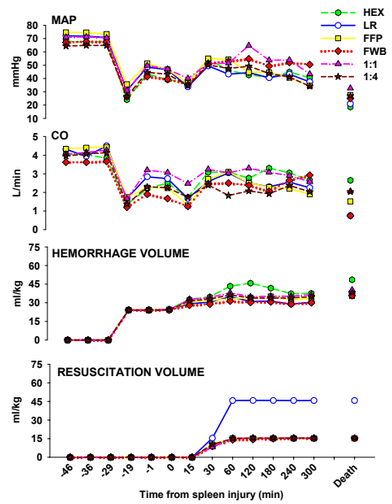


Figure 1 – Hemodynamics. The time course of the mean arterial pressure (MAP), cardiac output (CO), total hemorrhage volume (cumulative controlled and uncontrolled), and the resuscitation volume are shown. Until 15 minutes, all animals received the same treatments. Resuscitation was begun at 15 minutes after the spleen injury. Most of the colloid infusions were complete by 30 minutes, while the LR infusion required a longer time. These treatments resulted in similar hemodynamics outcomes.

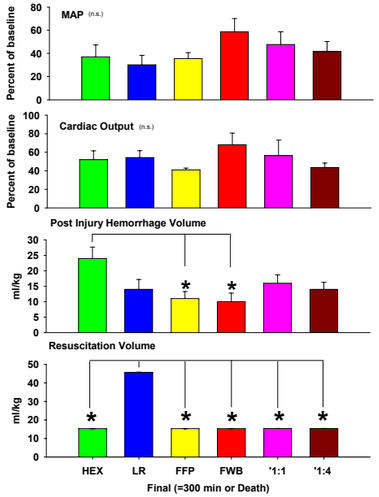


Figure 2 - A One Way ANOVA was performed on the final value (either 300 min in those that survived or the measurement taken at the time of the death sample), expressed as a percent of baseline. There were no statistically significant differences in the MAP and CO. The post spleen injury blood loss in the Hextend group (24 ± 3.7 ml/kg) was double the blood loss in the FFP (11 ± 2.3 ml/kg) and FWB (10 ± 2.8 ml/kg) groups despite their receiving the identical volumes and having similar hemodynamics. * $p < 0.05$. n.s. = not significant.

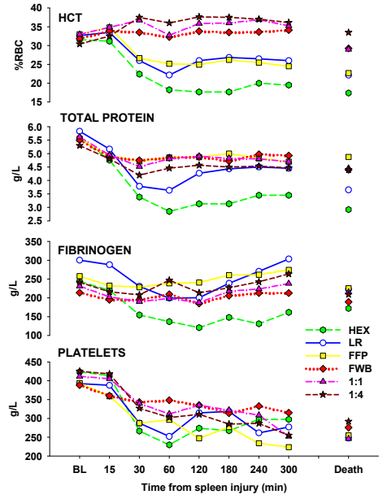


Figure 3 – The time course of the changes in hematocrit (HCT), plasma total protein and fibrinogen concentrations, and whole blood platelet concentration are shown. Despite getting the same volume of resuscitation, the HCT was lower in the Hextend group (minimum = 18%RBC) compared to FFP (25%) and LR (22%). The transient effect of the crystalloid LR on volume expansion is apparent in the HCT, protein, and fibrinogen responses.

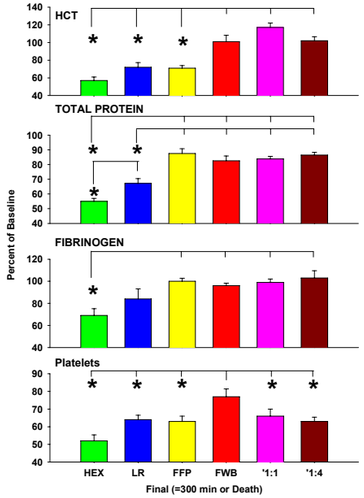


Figure 4 – As expected, the final value of the HCT was higher in the groups which received red blood cells compared to the FFP, LR, and Hextend. The total protein concentration was lower in the Hextend group compared to the others, including LR. It is known that Hextend results in 800 ml volume expansion *in vivo* for every 500 ml given, due to the fact that as the starch is metabolized, osmotically active particles are released (5), and these recruit fluid from the extravascular space to dilute the blood.

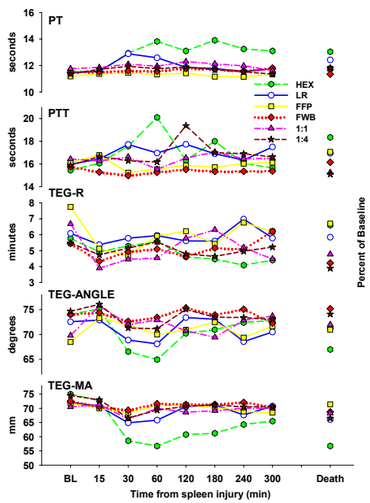


Figure 5 – Time course of the changes in prothrombin time (PT), activated partial thrombospondin time (PTT), and the thrombelastography (citrated blood activated with kaolin measured at body temperature).

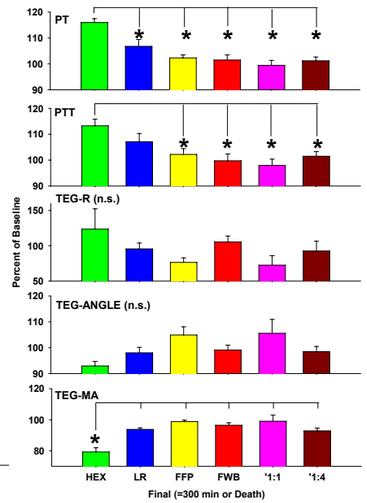


Figure 6 – The final values for the PT and PTT were significantly elevated in the Hextend group compared to the other groups received blood products or LR. The only parameter of the TEG that was significantly changed was the MA, indicating that the strength of the clot was reduced in the Hextend group, probably due to the greater dilutional effect it caused. * $p < 0.05$. n.s. = not significant.

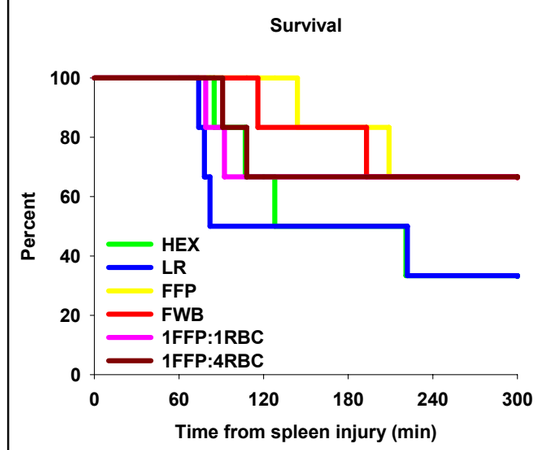


Figure 7 – Kaplan-Meier plot. There was 67% survival in all the groups that received blood products, while only a 33% survival in the groups receiving LR or Hextend. Despite losing a greater volume of blood, the survival for the Hextend group was equivalent to the LR group.

Conclusions

- Use of blood products as an initial resuscitation fluid preserved HCT, protein, and coagulation profile and prolonged survival, even when given at a fraction of the total blood loss (15.25 ml/kg resuscitation volume versus 35 – 40 ml/kg total hemorrhage volume in the blood product groups)
- An equal volume of Hextend caused a greater dilution of the coagulation factors and doubled the post-injury hemorrhage. The increase in PT did not reach those levels usually considered to be coagulopathic (i.e., INR = 1.5), yet was associated with a significant increase in hemorrhage. This model has demonstrated a direct relationship between uncontrolled hemorrhage blood loss and coagulation status.
- The FWB group (with high platelets) and the FFP groups (with high fibrinogen) had the lowest post-injury blood loss. Although the difference was not significant compared to the 1:1 and 1:4 component groups, it does suggest possible strategy towards developing the most effective hemostatic resuscitation fluid.
- The volume expansion effect of LR was short lived compared to Hextend. The metabolism of Hextend resulting in a very high hemodilution is not apparent in *in vitro* tests. While the hypo-coagulant effect on the PT, PTT, and TEG-MA by Hextend were consistent with the direct clot-formation interference found by others (4), we could equally explain them by the dilutional effect. The dose of Hextend used (15.25 ml/kg), exceeded the recommended maximum of 1000 ml (12.5 ml/kg in an 80 kg soldier) by the Tactical Combat Casualty Care paradigm, but only slightly (1220 ml given to an 80-kg soldier). This dose was selected to give equivalent volumes as would be in 4 units of FFP and PRBC to compare the survival benefit with the other groups.

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