Development of Intra-vital and Electron Microscopic Techniques for Assessment of Endothelial Glycocalyx in a Rat Hemorrhage Model.

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Introduction

Hemorrhage is the leading cause of death in the battlefield and the second leading cause of death in civilian trauma. The military recommends Hextend (6% hetastarch in Lactated Ringer's [LR] solution), as a small volume resuscitation fluid for pre-hospital use.

Recent work in our laboratory and others has found that there is higher survival when plasma is initially given instead of saline or Hextend, followed by packed red blood cells (PRBC). We think that plasma would support the endothelial function whereas saline and Hextend may disrupt the function of the lining (endothelium) of blood vessels.

The endothelium is lined with a special mesh-like layer of various proteins and sugars containing molecules called the endothelial glycocalyx (EG). This lining appears to affect vascular permeability. The two most promising techniques to quantify the structure and function of the delicate EG are electron microscopy (EM) and intra vital microscopy (IVM) which visualize the EG and microvascular flow.

Objectives

To develop intra-vital and electron microscopic techniques for assessment of endothelial glycocalyx in a rat hemorrhage model. This technique will be used to investigate if plasma resuscitation of severe hemorrhagic shock preserves the endothelial glycocalyx as compared with Lactated Ringer's, Hextend, or a 1:4 LR to packed red blood cell (PRBC) resuscitation.

Methods

Male Sprague-Dawley rats weighing 350-400 g were anesthetized with isoflurane. A cutaneous window was inserted through a skin incision on the dorsal area of the trunk over the *cutaneous maximus* muscle (figure 1). Then the rat was allowed to recover and instrumented the following day for hemorrhage experiments.

Figure 1. Cutaneous window

Methods, cont.

The left iliac artery and vein were catheterized for hemorrhage and infusion while the right iliac artery was catheterized with a telemetry device (Data Sciences International) for continuous monitoring of mean arterial pressure (MAP), heart rate (HR), pulse pressure, and core temperature. See figure 2 for timeline of events

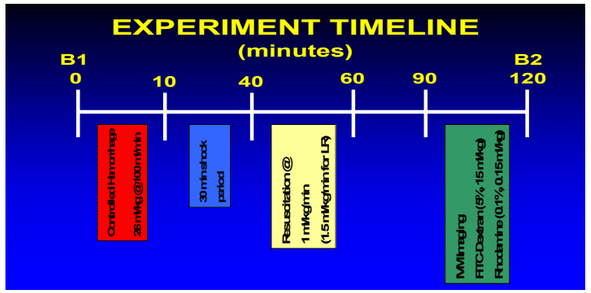

Figure 2. Experiment timeline

At time zero, 26 ml/kg blood was withdrawn manually in citrated (CPD) 1 ml syringes at a rate of 1 ml/min.

The first two mls were used for arterial blood gases (ABG), hematocrit (Hct), blood viscosity, and plasma protein measurements.

After a 30 min shock period, rats in the resuscitated groups were resuscitated. Eighty percent of the hemorrhage volume was replaced with a volume of each resuscitation fluid that will provide equivalent vascular expansion for each treatment: LR was 3 times the volume of the colloids.

IVM imaging was performed 30 minutes after resuscitation. FITC-dextran (5%, 15 mg/kg) and rhodamine (0.1%, 0.15 mg/kg) are injected IV then images of red and white blood cells of individual segments of the microvascular bed were recorded for analysis. At the 120 min time point, 2 mls of blood were taken for final analysis followed by a whole body fixation (figure 3).

Figure 3. Perfusion station

Results and Observations

We have designed and made a surgical platform that would slide in over the microscope platform after instrumentation (figure 4).

Figure 4. IVM Station

We were able to make video recordings of the vasculature with FITC-Dextran (figure 5) and Rhodamine (figure 6). We will be using the Simple PCI software to record and analyze the vascular recordings to determine WBC velocity for determination of leukocyte adhesion; ratio of fluorescence intensity from perivascular to intravascular areas; and measurement of microvessel diameter.

Figure 5. Vasculature under the window placed on the cutaneous maximus muscle using 5% FITC-Dextran (15 mg/kg BW) at 100x magnification.

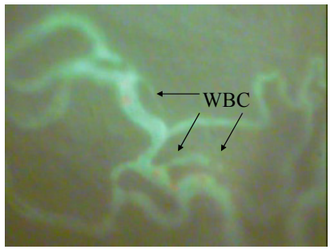

Figure 6. Vasculature of the same area using 0.1% Rhodamine (0.15 mg/kg BW) at 200x magnification

Results and Observations, cont.

We are currently using a less hazardous non arsenic based perfusion solution for EM and have added sodium nitrite, a vasodilator, in the perfusion flush solution for better perfusion of the microvasculature. The fixed tissues were sent to our collaborators at University of Texas San Antonio for EM (figures 7 and 8).

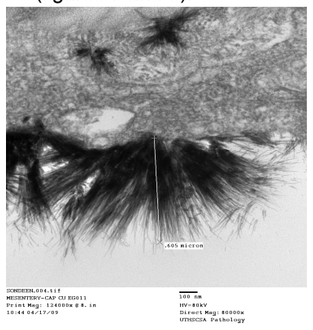

Figure 7. Glycocalyx on the mesentery capillary at 80000x magnification.

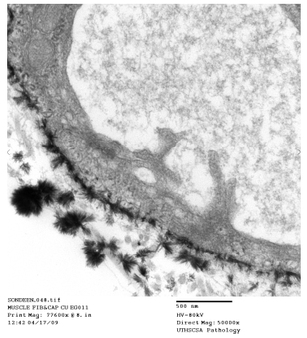

Figure 8. Glycocalyx on the muscle fiber and capillary at 50000x magnification.

Conclusion

We have established the methods needed to determine whether resuscitation with blood products preserves the EG and reduce capillary leak.

This will be the first study conducted to determine if some resuscitation fluids are better at preserving the EG and reducing capillary leak syndrome than other fluids following a 40% hemorrhage.

Acknowledgements

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References

1. Fresh whole blood is the best hypotensive resuscitative fluid in a severe hemorrhage pig model; American Heart association Circulation; 2005
2. The endothelial glycocalyx: composition, functions, and visualization.; Pflugers Arch; 454:345-359, 2007
3. Contrasting effects of colloid and crystalloid fluids on cardiac vascular permeability; Anesthesiology; 104:1223-1231, 2006