Treatment of Stored pRBCs with Amitriptyline Improves Lung Inflammation after Hemorrhagic Shock and Resuscitation in Mice

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Introduction: Anemia and hemorrhagic shock are leading causes of preventable death after traumatic injury. Studies have demonstrated the superiority of human blood product transfusion over crystalloid resuscitation, but use of aged units of packed red blood cells (pRBCs) can lead to pneumonia, sepsis, multi-organ failure, and death. Acid sphingomyelinase (Asm) is an enzyme on the erythrocyte membrane that has been shown to contribute to erythrocyte aging and microparticle formation, change that may contribute to lung inflammation after transfusion. We hypothesized that Asm inhibition in stored pRBCs would lead to decreased lung inflammation after hemorrhagic shock and pRBC transfusion.

Methods: Murine blood was collected stored as previously described with addition of either 125uM amitriptyline, a known Asm inhibitor, or vehicle alone (normal saline). For hemorrhagic shock and resuscitation, healthy male C57BL/6 mice underwent femoral artery cannulation were shocked to a mean arterial pressure of 25 ± 5 mmHg for 60 minutes and then resuscitated to a MAP of 75 ± 5 mmHg with fresh pRBCs or aged pRBCs treated with amitriptyline or vehicle. Animals were sacrificed and lung tissue was analyzed. H&E sections were given inflammation scores by a blinded observer and Gr1-positive cells were quantified to signify leukocyte infiltration. Values were averaged over 10 slides per mouse.

Results: Mice resuscitated with aged pRBCs demonstrated significantly higher levels of lung inflammation compared to those receiving fresh pRBCs. Mice receiving resuscitation with amitriptyline-treated aged pRBCs showed a significant decrease in lung inflammation as compared to vehicle-treated aged pRBCs. GR1-positive cell recruitment to the lungs showed similar results; aged pRBC transfusion led to a marked increase in GR1-positive cells in the lung compared to fresh pRBCs, and this effect was greatly reduced with amitriptyline-treated pRBC transfusion.

Conclusions: Our results suggest that storage of pRBCs with amitriptyline improves lung inflammation and leukocyte recruitment in a murine model of hemorrhagic shock and resuscitation. These results suggest that Asm inhibition in stored blood products has a potential role in the mitigation of lung injury seen in so many trauma patients receiving large volume pRBC transfusions.