

Microparticles from Stored Red Blood Cell Units Alter ZO-1 Expression in Endothelial Cells

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Introduction: Hemorrhagic shock is the most common cause of potentially preventable death after trauma. Packed red blood cells (pRBCs) are essential for resuscitation of patients with significant blood loss. During storage, pRBC units undergo a series of biological and physical changes known as the “red blood cell storage lesion.” In the clinical setting, transfusion of large volumes of older pRBC units is associated with increased complications, including lung injury.

An important component of the storage lesion is the shedding of microparticles, which are small extracellular vesicles that may be biologically active. Studies from our and other laboratories indicate that microparticles from stored red blood cells lead to endothelial cell activation, but the effect of these microparticles on endothelial tight junction proteins is unknown.

Hypothesis: We hypothesized that microparticles from stored red blood cell units would lead to decreased expression of the endothelial cell tight junction protein ZO-1.

Methods: Human pRBC units were obtained from our local blood bank at the end of the FDA regulated storage period (42 days) and microparticles were isolated by centrifugation. Confluent monolayers of human umbilical vein endothelial cells (HUVECs) were treated with microparticles or vehicle, then harvested and analyzed for ZO-1 expression by Western blotting or immunofluorescence and quantified with imaging software.

Results: Treatment of HUVECs with microparticles from pRBC units for four hours resulted in a dose-dependent reduction of ZO-1 protein as determined by Western blot (Figure) and immunofluorescence. Pre-treatment of microparticles with amitriptyline, which inhibits the enzyme acid sphingomyelinase, attenuated the decrease in ZO-1.

Conclusion: In the current study, microparticle treatment *in vitro* led to a dose-dependent decrease in ZO-1, a critical tight junction protein. This decrease was mitigated by acid sphingomyelinase inhibition. Microparticles from transfusion of stored pRBC units may lead to loss of the endothelial cell tight junction and contribute to morbidity after massive transfusion.

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