Lyophilized Platelets Show Hemostatic Function Independent of von Willebrand Factor

B. ISHLER, A. LEE, M. DICKERSON, and K. MOSKOWITZ

Dept. of Discovery and Research, Cellphire Inc, Rockville, MD

INTRODUCTION

von Willebrand Disease (vWD) is the most prevalent bleeding disorder, causing excessive bleeding and bruising in 1% of the population[1]. von Willebrand Factor (vWF) mediates platelet collagen interactions under high shear by binding to CD42b. Murine vWF does not interact with human CD42b[2]. Lyophilized human platelets (LHPs) are a platelet derived hemostatic agent under clinical development by Cellphire under the name Thrombosomes®.

AIMS

To determine the role, if any, that vWF plays in the promotion of hemostasis by LHPs.

METHODS

Surface expression of VWF binding sites in LHP and fresh platelets were measured by flow cytometry using a CD42b antibody.

The ristocetin cofactor assay was performed using an optical density aggregometer to measure ristocetin induced binding of vWF and subsequent agglutination of LHP.

The Total Thrombus-formation Analysis System (T-TAS) was used to assess thrombus formation by LHP under shear in collagen and tissue factor coated microcapillaries. Testing was performed in healthy and vWD plasma.

The hemostatic capability of LHP was assessed *in vivo* using NOD/SCID mice naturally expressing vWF that does not interact with the human vWF platelet receptor.

RESULTS

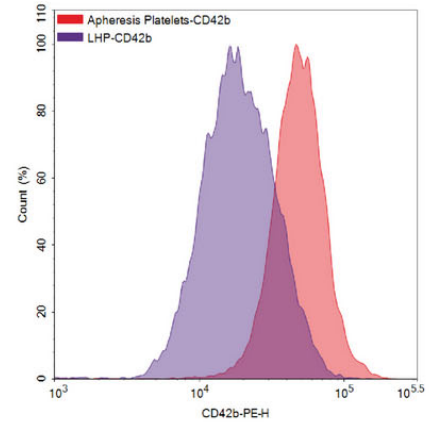

Figure 1: Fresh apheresis platelets and LHP were stained with an anti-CD42b antibody specific for the vWF binding site on the cell surface. Analysis by flow cytometry showed that expression of vWF binding sites was reduced by 55.6% in LHP relative to fresh platelets.

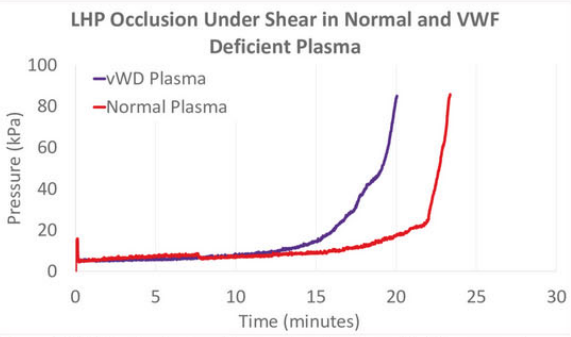

Figure 3: T-TAS occlusion to collagen under shear forces by LHP occurred similarly in normal and Type 3 vWD plasma as shown by pressure increases in the channel over time.

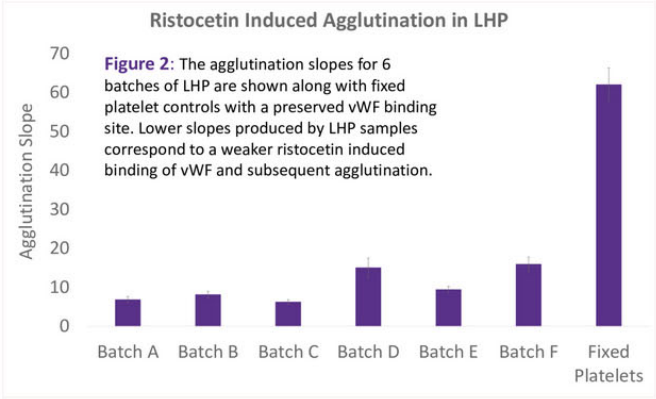

Figure 2: The agglutination slopes for 6 batches of LHP are shown along with fixed platelet controls with a preserved vWF binding site. Lower slopes produced by LHP samples correspond to a weaker ristocetin induced binding of vWF and subsequent agglutination.

Figure 4: Tail bleed clotting times in NOD/SCID mice demonstrate LHPs ability to promote hemostasis independent of vWF binding. Treatment with the high dose of LHP reduced bleeding times to 4.2 minutes relative to >9 minutes in untreated mice and mice administered human platelet rich plasma (PRP). Extended bleeding times in mice given human PRP is likely associated with the incompatibility between mouse vWF-A1 and human platelets.

CONCLUSIONS

LHP have reduced vWF binding sites on their surface, and appear to have hemostatic function independent of vWF *in vitro*. However, LHP can still support thrombus formation and hemostasis *in vivo*. This indicates that LHP may be used therapeutically to promote hemostasis independent of vWF in patients with vWD.

REFERENCES

1. Data and Statistics on von Willebrand Disease. Centers for Disease Control and Prevention; 2019 [cited 2020Jun26]. https://www.cdc.gov/ncbddd/vwd/data.html
2. Chen J, Tan K, Zhou H, Lo H-F, Roux DT-L, Liddington RC, et al. Modifying murine von Willebrand factor A1 domain for in vivo assessment of human platelet therapies. Nature Biotechnology. 2007Dec16; 26(1):114–9.

CONFLICTS OF INTEREST

All authors are employees of Cellphire, Inc. holding stock and/or stock options.

CONTACT INFORMATION

Presenting author may be reached at bishler@cellphire.com. Additional information about Cellphire may be found at www.cellphire.com.