Tetraglyme Plasma Treatment of Polyethylene Tubing Inhibits Platelet Activation: Flow Cytometry Studies

Lan Cao[1], Buddy D. Ratner[1,2], Thomas A. Horbett[1,2].
[1]Chemical Engineering, [2]Bioengineering, University of Washington, Seattle, WA, 98195.

Introduction:

Previous studies in our group have shown that tetraglyme plasma coatings have ultralow fibrinogen adsorption and greatly decreased platelet adhesion after exposure to blood *in vitro* or *ex vivo*. In addition, thrombin generation by the relatively few platelets that do adhere to tetraglyme surfaces is very low, suggesting a low degree of platelet activation. To determine whether tetraglyme surfaces might be activating platelets that are rapidly or readily shed and thus missed in our previous studies of activation of adherent studies, here we passed whole blood over the tetraglyme surface and measured activation of the non-adherent platelets by flow cytometry.

Methods:

Human blood was aseptically drawn into ACD VACUTAINER® tubes by venipuncture using a 20-gauge needle without use of a tourniquet. The first 2 mL of blood was discarded. Blood was drawn into 15 cm lengths of 3 mm I.D. tubing made of untreated polyethylene (PE), tetraglyme plasma treated PE (PE+Glyme), heparin benzalkonium chloride (HBAC) treated PE (PE+HBAC), medical grade polyurethane (PU), Tygon®, or Silastic®. The blood filled tubes were then attached to the platform of a shaker, and the tubes kept in contact with a 37°C water while shaken at 100 rpm or 200 rpm for 1 hour. 5 µl aliquots of blood from each tube were then mixed with 20µl of buffer containing a dye labeled antibody specific for one of the following antigens: CD61, CD62p and Pac-1. CD61 is a platelet specific and activation-independent marker whereas CD62p and Pac-1 are platelet activation-dependent markers. Dyes used were perCP(CD61), PE(CD62p), or FITC (Pac-1). After allowing 20 minutes at room temperature in the dark for cell staining, 1 mL of cold (4°C) 1% paraformaldehyde was added to the blood/dyed antibody mixture. Fixed cells were stored at 4° in the dark for 2-24 hours and then analyzed on the BD FACScan™ 3 Color Analyzer. All data were gated on CD61 perCP positive events.

Platelet adhesion on the lumenal surface of sample tubing was measured by assay of LDH released after detergent lysis. SEM analysis was also done and confirmed that there were no adherent red or white blood cells on the surfaces.

Results / Discussion:

As shown in Fig 1, platelets in blood contacted with HBAC treated PE showed the greatest CD62p and Pac-1 expression, at both shaking speeds. This was consistent with previous findings that heparin can activate platelets[1]. At the low shaking speed (Fig 1A), platelets from blood contacted with tetraglyme plasma coated PE and Tygon® showed the lowest CD62p and Pac-1 expression, so the

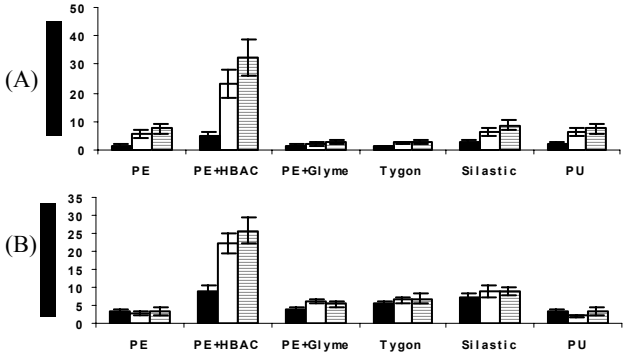

Figure 1. Percentage of platelets expressing activation-dependent antibodies normalized against values in resting blood. A, 100rpm; B, 200rpm. Black bar: CD62p+; white: Pac-1+; crossed, CD62p+/Pac-1+.

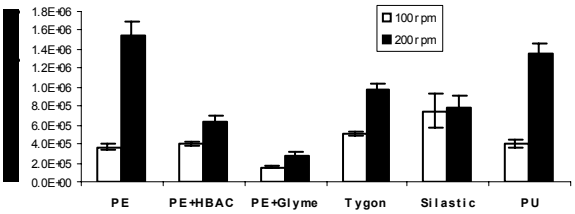

Figure 2. Platelet adhesion to the lumenal surface of 6 types of tubing.

activation of platelets was the least for these surfaces. However, at a higher shaking speed (Fig1B), tetraglyme plasma coated PE and Tygon® caused the highest activation. The reason for the increased activation at the higher shaking speed on these surfaces is not well understood. However, since flow cytometry only measures activation of platelets that remain in the bulk phase, if the tetraglyme surfaces adhere fewer activated platelets at the higher shaking speed than the control surface (PE), as suggested by Fig 2, the percentage of activated platelets in the bulk phase for tetraglyme would be larger than that on PE and other materials.

Conclusions:

Tetraglyme coated PE caused a lower degree of activation of the platelets that remained in the bulk phase than PE when blood filled tubes were shaken at 100 rpm, but activation was higher than PE when shaking was done at 200 rpm. To fully understand the significance of these results, it will be necessary to also measure activation of the surface adherent platelet population after exposure to whole blood.

Acknowledgements:

The work was supported by the NIH/NHLBI through grant # HL 67923.

References:

1. Warkentin et al, Heparin-induced thrombocytopenia: towards consensus, Thromb. Haemostas, 1998, 79(1), 1-7.