Demonstrating GPIbα-thrombin interaction in procoagulant platelet formation leads to identification of potential specific inhibitors

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Procoagulant platelets are a highly activated platelets-subpopulation which support thrombin generation. We previously reported that coronary artery disease (CAD) patients have excess procoagulant platelet response to thrombin. Thrombin directly activates platelets via cleavage of PAR1 and PAR4, but is also known to interact with GPIbα via exosite II.

**Aim:** To understand the mechanism of thrombin induced procoagulant platelets and to generate novel antplatelet therapies.

**Hypothesis:** Involvement of both PAR-dependent and GPIbα-thrombin interaction pathways.

**Methods:** Procoagulant platelets detected by FACS (GSAO, P-selectin, lactadherin) in whole blood and washed platelets after treatment with PAR, GPIbα, exosite II inhibitors and heparins prior to thrombin stimulation.

**Results:** Preincubation with combined PAR1 and PAR4 inhibitors reduced procoagulant platelet formation by 40±5.7%, indicating PAR pathways are not sufficient for thrombin effect. Thrombin exosite II was implicated by reduction of thrombin induced procoagulant platelets by exosite II targeting aptamer (92±4.1%) and heparin. Interaction with GPIbα was demonstrated using competition studies with recombinant soluble GPIbα, glycolocalcin (49±7.7%). Involvement of the N-terminus of GPIbα was implied by use of NK protease which cleaves GPIbα proximal to the thrombin binding site (39.5±2.9%). Additive inhibition of procoagulant platelet formation after thrombin stimulation was achieved by combination of GPIbα cleavage and PAR1 and PAR4 inhibition. Having demonstrated a role for displacement of thrombin from GPIbα in targeting procoagulant platelet formation, we evaluated the effect of potential inhibitors of this binding on procoagulant platelet formation in healthy controls and CAD patients and one (inhibitor X) resulted in selective targeting of procoagulant platelets (40.5±10% and 40.6±9.5% respectively).

**Conclusion:** The GPIbα/thrombin exosite II interaction is strongly implicated in thrombin induced procoagulant platelet formation. We identify a potential therapeutic that specifically targets procoagulant platelets as a possible adjunct anti-platelet therapy in CAD. We aim to confirm our results ex vivo and in vivo by using PAR4 knockout and GPIb mutated mice.

**Figure 1:** Whole blood was treated with PAR1 and PAR4 inhibitors or exosite II aptamer and washed platelets were treated with heparin, glycolocalcin, NK protease or NK and PAR inhibitors prior to thrombin stimulation (2 U/mL).

**Figure 2:** Whole blood from healthy donors (n=4) or CAD patients (n=10) was preincubated with control or inhibitor X (73 ug/ml) prior to thrombin stimulation (2 U/mL). Data are expressed as fold change from baseline (*p<0.05, ***p<0.001).