405. The role of platelet-attached glycans in platelet function

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Background:
The platelet membrane contains many highly branched carbohydrate chains; which are capped by sialic acid. These glycans can be cleaved off the surface and this has been implicated in the clearance of senescent and cold-stored platelets, as well as in immune thrombocytopaenia patients. The majority of glycans are attached to one of the main platelet adhesion receptors, glycoprotein (GP)Ibα. So far, four different forms of the enzyme responsible for cleaving sialic acid have been identified in mammals (neuraminidases, NEU1-4); however their role in platelet function is largely unknown.

Aim:
To study the potential role of glycans and neuraminidases in platelets.

Method:
Donors were consented to donate either whole-blood (to obtain PRP) or apheresis platelets (n=8). Platelet rich plasma (PRP) was stimulated with ristocetin, ADP and arachidonic acid (n=6). NEU1 and NEU2 membrane expression was measured by flow cytometry, as were platelet-attached glycans using *Ricinus Communis Agglutinin*-1 (RCA-1; detecting galactose) and Wheat Germ Agglutinin (WGA; detecting sialic acid and N-acetyl-D-glucosamine, GlcNAc). GPIIb/IIIa-integrin and/or GPIbα mediated signalling was inhibited by RGDS, addition of GlcNAc or *O*-sialo-glyco-endopeptidase cleavage respectively. Apheresis platelets were studied on day 1, 2, 5, 7, 9 post-collection.

Result:
Activation of GPIbα by ristocetin induced a 3-fold increase in RCA-1 binding ($p<0.05$), and reduced WGA binding ($p<0.05$), while stimulation by ADP or AA showed no effect. Interestingly, basal membrane expression of both NEU1 and 2 was found, which increased by 5- and 3-fold respectively following ristocetin stimulation ($p<0.05$). Inhibition of GPIIb/IIIa-integrin inhibited NEU1 expression. More importantly, GPIbα inhibition and/or cleavage of its extracellular part decreased the majority of membrane-associated NEU1 and NEU2. In apheresis platelets, ristocetin stimulation increased cleavage of sialic acid significantly, and was found to be highly variable between donors.

Conclusion:
These results show a potential novel role for NEU1 and NEU2 in platelet activation, which is highly dependent on GPIbα–mediated signalling.