

Increasing Shear Rates Cause Shedding Of Platelet Glycoprotein (GP)VI

Elizabeth Gardiner,¹ Jane Arthur,¹ Jing Jing,¹ Justin Hamilton,² Michael Berndt,¹ Robert Andrews.¹ ¹Department of Immunology, Monash University, Melbourne, Australia; ² Australian Centre for Blood Diseases, Monash University, Melbourne, Australia

Glycoprotein (GP)VI, which binds collagen, and GPIb-IX-V, which binds von Willebrand factor (vWF) and other ligands, form a unique adhesion-signalling complex on human platelets. Following vascular damage or disease, engagement of GPVI/GPIb-IX-V leads to $\alpha_{IIb}\beta_3$ -dependent thrombus formation. We previously showed ligand binding to GPVI leads to metalloproteinase-dependent ectodomain shedding, generating an ~55-kDa soluble GPVI fragment and an ~10-kDa remnant fragment that remained membrane-associated.

Aim To determine whether shear force was sufficient to induce shedding of GPVI.

Methods Human platelet-rich plasma or washed platelets were subjected to increasing shear rates in a cone-plate viscometer and then levels of intact and cleaved GPVI were examined by western blot using anti-GPVI antibodies raised against either the GPVI ectodomain (recognising intact and ~55-kDa soluble GPVI) or the GPVI cytoplasmic tail (recognising intact and ~10-kDa remnant GPVI).

Results Increasing platelet aggregation was observed in platelet suspensions subjected to shear rates from 300 s^{-1} up to 3000 s^{-1} for 5 minutes and >90% aggregation was achieved using a shear rate of $10,000\text{ s}^{-1}$. Aggregation was blocked by inclusion of $10\text{ }\mu\text{g/ml}$ function blocking anti- $\alpha_{IIb}\beta_3$ (CRC64). Increasing shear rates also induced a loss of full length GPVI and the appearance of the ~55-kDa soluble GPVI ectodomain and increasing levels of the ~10-kDa GPVI remnant on platelets, and 5 to 7-fold increase in soluble GPVI in plasma by ELISA. Proteolysis of GPVI was blocked by the metalloproteinase inhibitor, GM6001, implying that shearing of platelets was sufficient to cause activation of platelet metalloproteinases, in the absence of GPVI ligands. Preliminary data further suggested that blockade of $\alpha_{IIb}\beta_3$, GPIb α or vWF minimally affects shear-induced shedding of GPVI. **Conclusions** Together, these results suggest GPVI shedding is triggered in shear-activated platelets, with potential implications for the stability of a forming thrombus at arterial shear rates. No conflict of interest to disclose.