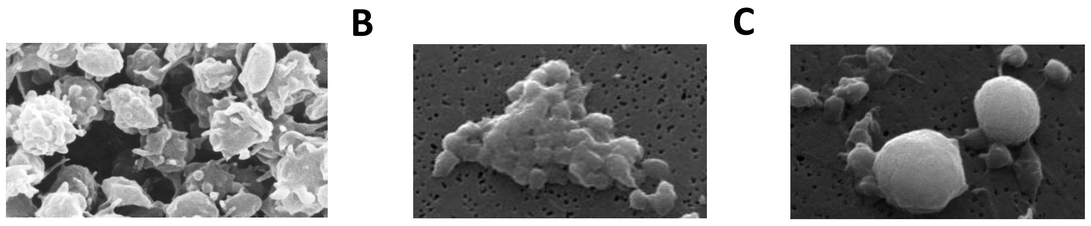
Platelet aggregation and activation assessed in small volumes of fixed whole blood – high throughput assays for testing the effects of pharmacological compounds on platelet function

Natalia Dovlatova[1], Ann White[1], Andrew Johnson[1], Sue Fox[2], Mohammad Algahtani[2], Jane May[2], Stan Heptinstall[1,2]

[1]Platelet Services Ltd, Nottingham, UK; [2]University of Nottingham, UK

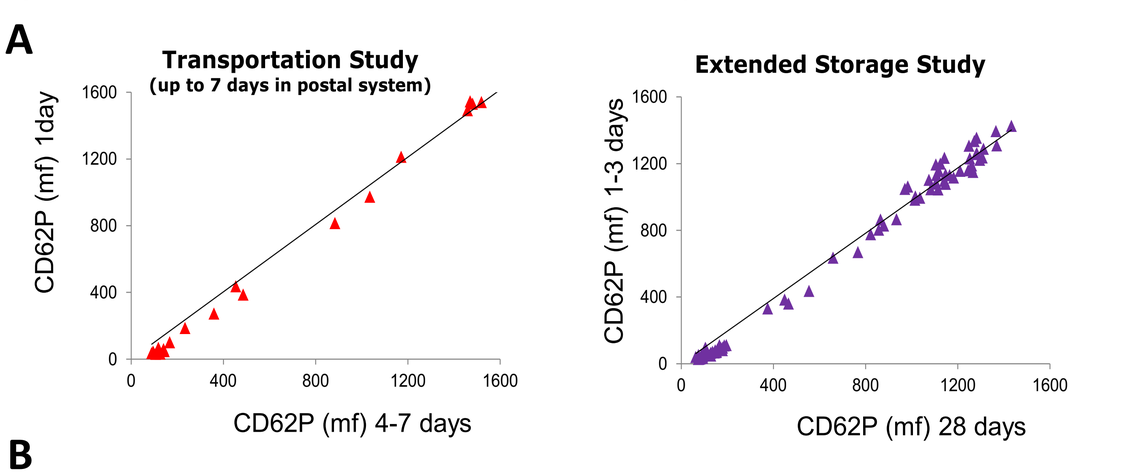
Background. Platelet function is usually studied in freshly prepared whole blood (WB) or platelet rich plasma (PRP) and assessed within 2-4 hours of obtaining the blood sample. Platelet stimulation in vitro leads to platelet activation (A), aggregation (B) and formation of platelet-leucocyte conjugates (PLC) (C). The application of fixatives specifically developed for stabilising platelet activation markers (PAMFix) and platelet aggregates and PLCs (AGGFix) allows the measurement of platelet reactivity to be performed at a later stage.

A B C

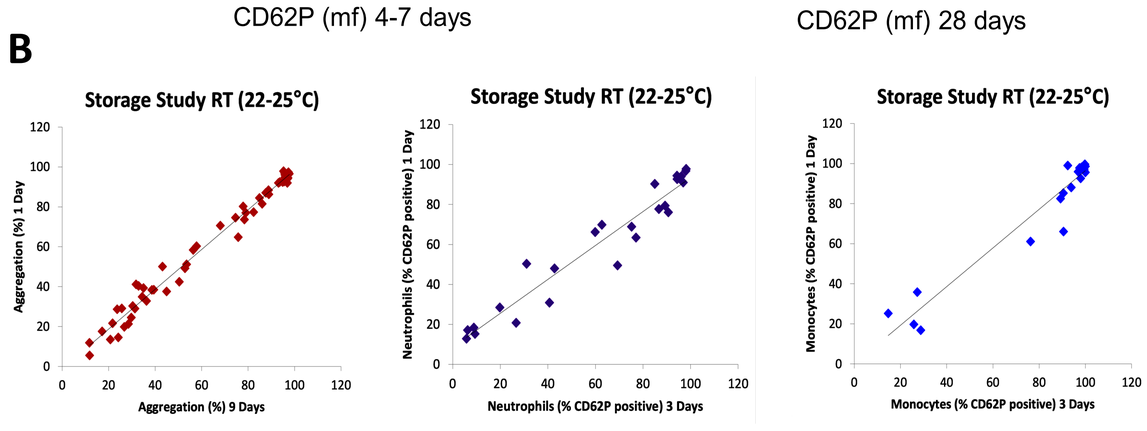


Results 1. Levels of P-selectin (CD62P) on platelets in WB were determined at different times after platelet stimulation and addition of PAMFix; excellent stability was demonstrated, first up to 9 days, and later up to 28 days (A). Similarly, measurements of platelet aggregation were stable for at least 9 days after adding AGGFix to blood samples following platelet stimulation with a variety of agonists; PLC was stable for at least 3 days (B).

A



B

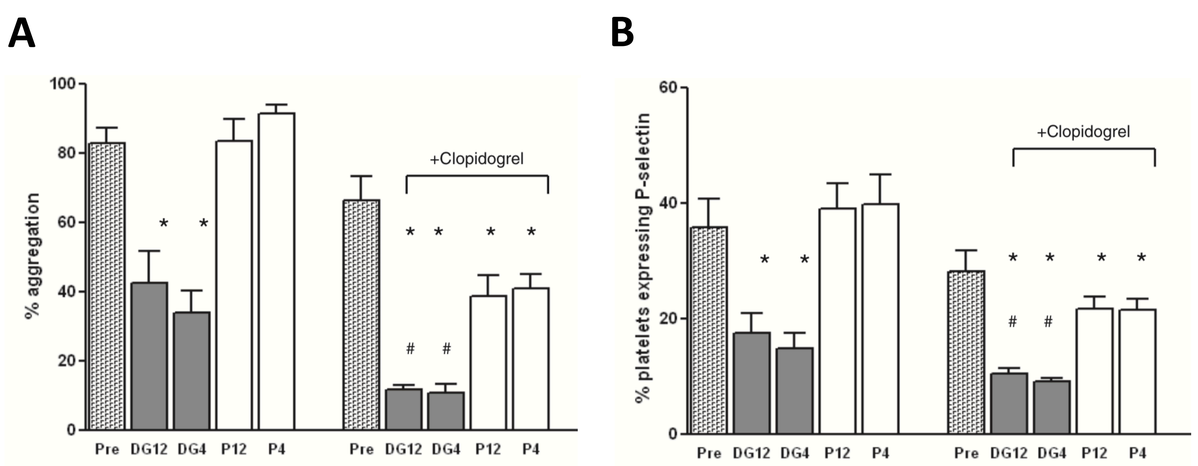


Conclusions. Assessing several measures of platelet function within a small volume of fixed whole blood sample, stabilised with dedicated fixatives, can offer a high throughput screening approach to test the effects of multiple compounds on platelets.

Methods. Platelets in whole blood (WB) were stimulated with a range of different platelet agonists following which PAMFix or AGGFix was added and the platelets were analysed using flow cytometry at a later stage (within 9 days). Platelet activation was assessed as the level of P-selectin (CD62P) expression, platelet aggregation was measured by platelet counting (% fall in number of single platelets) and PLC was quantitated using flow cytometry (% CD62P +ve monocytes and neutrophils).

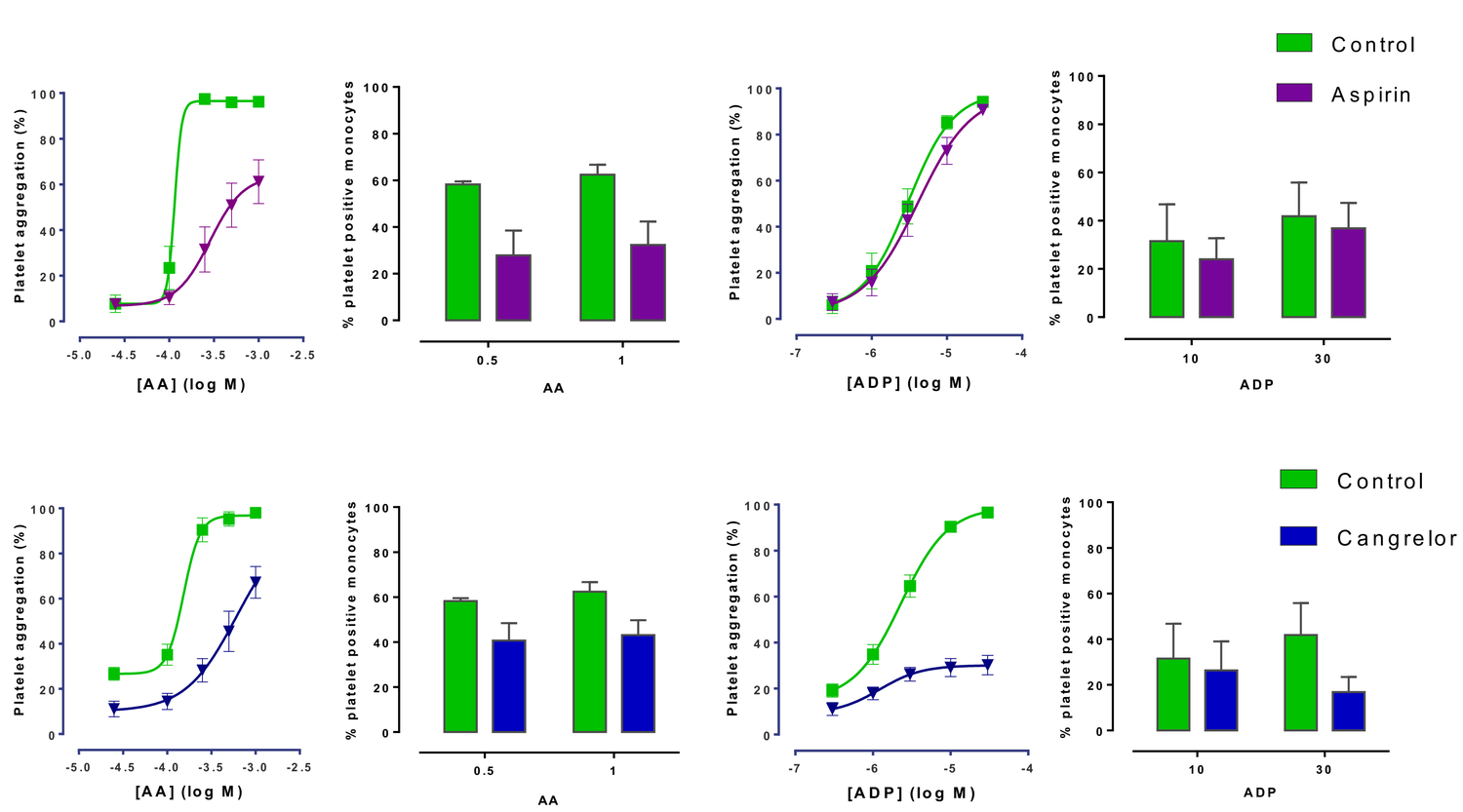
Results 2. AGGFix was used in a study to investigate the effects on platelet aggregation of an EP3 receptor antagonist, DG-041, administered to healthy volunteers with and without clopidogrel. DG-041 inhibited platelet aggregation and clopidogrel added to this inhibition (A). Platelet P-selectin was measured in parallel and both approaches yielded very similar data (B).

A B



Samples were stimulated with U46619 (thromboxane analogue) and PGE2; *p < 0.05 compared with Pre within each treatment group; #p < 0.05 for DG041 (DG) treatment groups compared with the corresponding placebo (P) group.

Results 3. Platelet activation, aggregation and PLC formation were studied in a 96-well plate format. The wells of a flat bottom plate were coated with agonists and small aliquots of WB treated with various inhibitors were added to each well. The plate was shaken at 1000rpm for 5 mins at 37°C, after which AGGFix was added, thus fixing platelet aggregates and PLCs. Aggregation and PLC formation were assessed in the same small volume sample and results were in line with the expected actions of agonists and inhibitors. For assessing platelet activation, the samples were stimulated without shaking and contained EDTA to prevent platelet aggregation. Multiple platelet inhibitors were used at a range of concentrations providing an IC50 measure for each compound (data not shown).



E: Info@plateletservices.com | www.plateletservices.com