Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol16Issue1abst090P.pdf

Dichotomy in Platelet Functions: Inhibition of platelet $P2Y_1$-dependent cell motility is independent of classical $P2Y_1$ receptor signalling pathways

R. Amison, C. Page, S. Pitchford. Institute of Pharmaceutical Sciences, King's College London, London, UNITED KINGDOM.

Rationale: Platelets express $P2Y_1$ (1), which when activated by ADP induces the formation of platelet-leukocyte complexes and drives leukocyte recruitment to inflammatory tissues through RhoA activation (2). Platelets are also necessary for pulmonary leukocyte recruitment after LPS challenge (3). However, under these conditions no platelet aggregation is observed. We therefore investigated the signaling cascades involved in platelet activation to both hemostatic and inflammatory stimuli using specific inhibitors for PLC (U73122) signaling downstream of $P2Y_1$ and inhibitors to cdc42 (ML141), Rac1 (NSC23766) and ROCK (GSK429286).

Methods: Platelets and neutrophils were isolated from human venous blood. PRP was treated with 1, 10 and 100µM of U73122, ML141, GSK429286 or 0.1, 1 and 10µM NSC23766 prior to stimulation with 10µM ADP and aggregation assessed. $1x10^8$ platelets/ml were incubated with each antagonist and stimulated with 100nM ADP and chemotaxis quantified towards 30nM f-MLP in a transwell chemotaxis assay. Finally antagonist treated platelets were stimulated with 100nM ADP and incubated with $5x10^6$/ml isolated neutrophils. Neutrophil chemotaxis towards 100nM MDC was then quantified. Data were analysed by 1-way ANOVA followed by Dunnet multiple comparison post-test. $P<0.05$ was considered significant.

R**esults:** Inhibition of platelet PLC significantly inhibited ADP induced platelet aggregation (10µM: 49.89±7.17% $P<0.001$, 100µM: 2.07±3.03% $P<0.001$ compared to vehicle (72.39±7.17%) however no inhibition was observed with either cdc42 (68.32±9.12%), Rac1 (73.30±5.41%) or ROCK (73.19±5.51%). In contrast, platelet chemotaxis towards 30nM f-MLP (2.7±0.2) was significantly attenuated by cdc42 (100µM: 1.50±0.05, $P<0.001$), Rac1 (10µM: 1.51±0.43, $P<0.05$) and ROCK inhibition (100µM: 1.05±0.21 $P < 0.05$) but not through PLC inhibition (2.72±0.38). Platelet-induced neutrophil chemotaxis towards MDC (2.72±0.30) was also significantly inhibited by cdc42 (100µM: 1.17±0.32, $P<0.01$), Rac1 (100µM: 1.22±0.13, $P<0.05$) and ROCK (100µM: 1.32±0.36, $P<0.05$), but not PLC treatment (2.41±0.30).

Conclusions: This data reveals that whilst PLC inhibition inhibits ADP induced platelet aggregation, it is not involved in platelet motility or platelet induced neutrophil chemotaxis assays previously been shown to be $P2Y_1$ dependent in our group. Inhibition of platelet chemotaxis and platelet neutrophil chemotaxis however was inhibited by cdc42, Rac1 and ROCK blockade. This suggests that $P2Y_1$ receptor activation under inflammatory conditions might signal through alternate pathways involving Rho GTPases in contrast to classical $P2Y_1$ signaling through PLC, suggesting that platelet signaling is selective towards a dichotomy in platelet function 1. Nylander,S. *et al.* (2003) *Thromb Res.* **111**, 65-73 2. Amison,R. *et al.* (2015): *J. Allergcy Clin. Immunol*. **135(2)**, 528-538 3. Pan,D. *et al.* (2015): *Blood.* **125(7)**, 1146-58