Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol18Issue1abst077P.pdf

Anti-thrombotic efficacy of cyclic nucleotide modulators in combination with a $P2Y_{12}$ inhibitor

P. C. Armstrong[1], P. Ferreira[1], M. V. Chan[1], C. Shih[2], M. H. Lundberg Slingsby[1], T. D. Warner[1]. [1]Blizard Institute, Queen Mary University of London, London, United Kingdom, [2]Pharmacology, National Defence Medical Centre, Taipei, Taiwan.

Background: Endothelium derived prostacyclin and nitric oxide act upon platelets stimulating, respectively, adenylate cyclase and guanylyl cyclase to elevate intra-platelet levels of the cyclic nucleotides cAMP and cGMP and so maintain platelet quiescence. We, and others, have previously demonstrated a synergistic relationship exists between cyclic nucleotides and $P2Y_{12}$ inhibition (1-3). Drugs that modulate soluble guanylate cyclase or those that inhibit phosphodiesterase (PDE), targeting cyclic nucleotide generation and degradation respectively, are approved treatments for pulmonary hypertension. We therefore sought to determine *in vivo* and *ex vivo* the anti-thrombotic efficacy of these approved drugs in combination with a $P2Y_{12}$ inhibitor.

Methods: Mice (C57Bl6) were administered sub-optimal doses of prasugrel (0.3mg/kg i.v; vehicle 0.2%DMSO) or cinaciguat (0.3mg/kg i.v; vehicle 2% DMSO) plus dipyridamole (2.0mg/kg i.p; vehicle 0.01M HCL), or combination of all three (PCD). Mice were anaesthetized (ketamine 100mg/kg & xylazine 20mg/kg, i.p) and blood taken from the inferior *vena cava* into lepirudin (0.25mg/ml final). Whole blood aggregometery (4) was performed using as agonists arachidonic acid (1mM, AA), collagen (10µg/ml), a PAR-4 receptor agonist GYPGKF (30µM) or thromboxane A_2 mimetic U46619 (3µM). Alternatively, anaesthetised mice underwent ferric chloride (10%)-induced thrombosis of carotid artery and blood flow was monitored to determine the time to occlusion, defined as cessation of blood flow for 60seconds. Data presented as mean s.e.m. and compared using t-test or one-way ANOVA with Tukey post-hoc test, as appropriate. Studies and analysis were not randomised or blinded.

Results: Blood from mice treated with PCD demonstrated significantly lower aggregatory responses than blood from vehicle treated animals (AA, 62±10% vs 22±16%; collagen, 53±9% vs 22±9%; GYPGKF, 37±9% vs 7±4%; U46619, 48±11% vs 13±6%; $p<0.05$ for all, n=5-8). *In vivo* thrombotic response was not altered from that in mice treated with vehicle (432±50seconds) by prasugrel (438±87seconds) or cinaciguat plus dipyridamole (482±110seconds). In contrast, PCD significantly caused a significant increase in the time to occlusion (1472±210seconds; $p<0.05$, n=6 for all).

Conclusion: Our studies demonstrate that cinaciguat and dipyridamole when combined with prasugrel produce strong anti-platelet effects *in vivo*. We therefore provide proof of concept that combination of approved drugs at low doses to modulate cyclic nucleotide levels alongside $P2Y_{12}$ inhibition produces a novel anti-thrombotic therapeutic regimen.

References:

1) Cattaneo *et al*. (2007). *JTH* **5**: 577-82.
2) Kirkby *et al*. (2013), *Proc Natl Acad Sci U S A*, **110**: 15782-7
3) Chan *et al*. (2016). *Br J Clin Pharmacol*, **81**: 621-33
4) Armstrong *et al*. (2015). *Blood* **126**: e11-18.