Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol13Issue3abst228P.pdf

Effect Of Platelet Turnover On Efficacy Of Dual Aspirin-Prasugrel Therapy In Mouse Carotid Artery Injury Model

INTRODUCTION: Currently it is common practice to prescribe the cyclo-oxygenase (COX) inhibitor, aspirin, in conjunction with an antagonist of platelet ADP $P2Y_{12}$ receptors, such as clopidogrel or prasugrel, for secondary protection against thrombotic vascular events. In presence of irreversible inhibitors platelet responses can only return as newly formed platelets enter the circulation and we have recently demonstrated *in vitro* how newly formed drug-free platelets can act as seeds for aggregate formation during anti-platelet therapy (1). Importantly, elevated platelet turnover is associated with reduced efficacy of anti-platelet medication and increased cardiovascular events. We therefore examined the effect of platelet turnover on thrombotic response following aspirin and prasugrel treatment in mice using an *in vivo* arterial thrombosis model.

METHODS: C57Bl/6 wild type (WT) mice were purchased from Charles River UK. All mice were aged 8-12 weeks (20-25g) and housed for a minimum of 7 days before commencement of experiments. They were housed on a 12-hour light-dark cycle, at a temperature of 22-24°C with access to water and food *ad libitum*. Animal procedures were conducted in accordance with Home Office legislation under "The Animals (Scientific Procedures) Act 1986" and were subject to local approval from Queen Mary University of London and Imperial College London Ethical Review Panels. Platelet turnover was confirmed by injecting mice with an anti-platelet (CD41) antibody conjugated to alexa-488 fluorophore (EMFRET, Germany) and subsequent quantification by flow cytometry. Mice received a single dose of aspirin (100mg/kg; *i.v.*) and prasugrel (10mg/kg; *i.v*) either 2, 24, 48 or 72 hours before being anaesthetised with ketamine (100mg/kg) & xylazine (10mg/kg) *i.p.* The carotid artery was then exposed and isolated from surrounding tissues to permit a ferric chloride (10% solution) soaked filter paper to be applied to its surface for 3 mins. The carotid artery was then flooded with saline and the filter paper removed. A doppler flow probe (Transonic, USA) was then placed around the artery and flow monitored over the proceeding 30 mins. The time to stable occlusion (defined as flow 0.0±0.2ml/min for 1 minute) was recorded. Each experimental group consisted of 4 mice. Data are presented as mean ±s.e.m and were analysed using one-way ANOVA with Tukey post-hoc test.

RESULTS: We initially confirmed previous observations that complete platelet turnover in mice occurs every 5 days, with approximately 20% of total platelets replaced each day. In vehicle-treated mice, vessel occlusion occurred after 356±25 secs. In contrast, no occlusion occurred in mice treated with aspirin and prasugrel 2 hours previously. In 3 out of 4 mice examined 24 hours after drug treatment the thrombotic response had returned to normal (occlusion at 476±96 secs, $p<0.05$ versus t=2 hours); 72 hours following treatment, occlusion occurred after 480±145 secs (n=4).

CONCLUSION: This data indicates that the anti-thrombotic efficacy of combined aspirin and prasugrel is significantly blunted following a 20% renewal of platelets. Consideration of such phenomena, particularly in conditions associated with increased platelet turnover, may provide some explanation for the variations in the anti-platelet effects of these drugs.

REFERENCE:

(1) Hoefer T *et al*. (2015) Arterioscler Thromb Vasc Biol. **35**: 2122-2133