**P2Y<sub>12</sub> receptor blockade potentiates the anti-platelet effects of both prostacyclin and nitric oxide**

Nicholas S. Kirkby<sup>1,2</sup>, Rachit Singhal<sup>1</sup>, Jane A. Mitchell<sup>2</sup>, Timothy D. Warner<sup>1</sup>

<sup>1</sup>William Harvey Research Institute, Barts & the London School of Medicine, London EC1M 6BQ, United Kingdom, <sup>2</sup>National Heart & Lung Institute, Imperial College, London SW3 6LY, United Kingdom

ADP is an important mediator of secondary platelet aggregation acting via P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors. Activation of P2Y<sub>12</sub> receptors, the target of thienopyridine anti-thrombotic drugs, inhibits adenylyl cyclase and so promotes aggregation by reducing intraplatelet levels of cAMP. Prostacyclin (PGI<sub>2</sub>) increases cAMP, which both directly, and in synergy with NO-stimulated cGMP, suppresses platelet reactivity. As blockade of P2Y<sub>12</sub> receptors has been reported to sensitize platelets to the effects of PGI<sub>2</sub> we hypothesised that blockade of P2Y<sub>12</sub> would also sensitize platelets to inhibition by NO.

Using light transmission aggregometry we assessed the inhibitory effects of PGI<sub>2</sub> (0.3-100nM) or the NO donor DEA/NONOate (0.1nM-10µM) upon the aggregation of human washed platelets induced by thrombin (0.01-1U/ml) in the presence or absence of the P2Y<sub>12</sub> receptor blocker prasugrel active metabolite (PAM; 3µM). In parallel experiments platelet levels of cAMP and cGMP were measured rather than aggregation (n=5 for all).

P2Y<sub>12</sub> blockade reduced sensitivity (-logEC<sub>50</sub>: vehicle, 0.80±0.04; PAM, 0.37±0.05; p=0.0002) but not maximal responses to thrombin (E<sub>max</sub>: vehicle, 59±2%; PAM, 61±4%; p=0.6). PGI<sub>2</sub> caused concentration-dependent inhibition of aggregation induced by a maximal concentration of thrombin (-logEC<sub>50</sub>: 8.1±0.1), and this was potentiated by PAM (-logEC<sub>50</sub>: 9.0±0.1; p<0.0001). PGI<sub>2</sub> increased platelet cAMP content (E<sub>max</sub>: 5.1±0.4pmol), but less so in the presence of thrombin (E<sub>max</sub>: 3.0±0.3pmol; p<0.05). In the presence of PAM, the effects of PGI<sub>2</sub> on cAMP were augmented (E<sub>max</sub>: 7.4±0.8pmol) and the inhibitory effects of thrombin were blocked (E<sub>max</sub>: 7.6±0.7pmol; p<0.05). DEA/NONOate also inhibited platelet aggregation (-logEC<sub>50</sub>: 7.0±0.3) and this was enhanced in the presence of PAM (-logEC<sub>50</sub>: 8.3±0.2; p=0.005). cGMP levels were increased by DEA/NONOate, but were unaffected by thrombin or PAM. (The same pattern of responses was found in matching studies using platelet-rich plasma and measurement of platelet aggregation in 96-well plates).

P2Y<sub>12</sub> blockade potentiates the inhibitory effects of PGI<sub>2</sub> on platelet aggregation, probably by preventing agonist-induced inhibition of adenylyl cyclase. The anti-platelet effects of NO are also enhanced by P2Y<sub>12</sub> blockade without increase in the levels of cGMP. This may be explained by synergy between cGMP and increased cAMP following from P2Y<sub>12</sub> blockade.