P2Y12 receptor blockade potentiates the anti-platelet effects of prostacyclin and nitric oxide

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ADP is an important mediator of secondary platelet aggregation, which acts via P2Y1 and P2Y12 receptors, the target of the thienopyridine anti-platelet drugs (clopidorel). P2Y12 activation inhibits adenylate cyclase and facilitates aggregation by reducing inhibitory cAMP. In vivo, prostacyclin (PGI2) increases cAMP, which, both directly, and in synergy with NO-stimulated cGMP, serves to supress platelet reactivity. We hypothesised that blockade of P2Y12 may exert its anti-platelet effect in part by sensitising platelets to the inhibitory effects of PGI2 and NO.

Blood was collected from healthy human volunteers (n=5) and platelets isolated by centrifugation. Platelets were washed with modified Tyrodes buffer and treated prasugrel-active metabolite (PAM; 3uM; P2Y12 antagonist) or vehicle. Light transmission aggregometry was used to determine responses to thrombin (0.01-1U/ml) and the ability of PGI2 (0.3-100nM) and DEA/NONOate (0.1nM-10uM) to inhibit thrombin (1U/ml)-induced aggregation. In parallel, washed platelets treated with PAM or vehicle, were incubated with PGI2 or DEA/NONOate with or without thrombin, and [cAMP] and [cGMP] measured. Aggregation data were analysed by t-test and [cAMP] and [cGMP] data by 1-way ANOVA.

P2Y12 blockade reduced sensitivity but not maximal responses to thrombin (E_max vehicle: 59.2±1.5%, PAM: 61.3±4.0%; p=0.635). PGI2 caused concentration-dependent inhibition of thrombin-induced aggregation (-logEC50: 8.07±0.06), which was potentiated by PAM (-logEC50: 8.96±0.05; p<0.0001). PGI2 increased platelet cAMP content (E_max: 5.09±0.39pmoles) and this was reduced by thrombin (E_max: 3.03±0.29pmoles; p<0.05). In the presence of PAM, the effects of PGI2 on cAMP were augmented (E_max: 7.40±0.75pmoles) and were not altered by thrombin (E_max: 7.63±0.66pmoles; p<0.05). DEA/NONOate also inhibited platelet aggregation (-logEC50: 7.00±0.29) and this was enhanced in the presence of PAM (-logEC50: 8.31±0.17; p=0.0052). [cGMP] was increased by DEA/NONOate, but this was not altered by thrombin or PAM. Similar aggregation data were obtained in platelet-rich plasma using 96 well plate aggregometry.

P2Y12 blockade potentiates the inhibitory effects of PGI2 on platelet aggregation, probably by preventing agonist-induced reductions in [cAMP]. P2Y12 blockade enhances the anti-platelet effects of DEA/NONOate to a similar extent but this is not accompanied by changes in [cGMP]. As such, this may reflect synergy between NO-induced cGMP and the increased levels of cAMP that follow P2Y12 blockade.