P2Y12 INHIBITION GREATLY POTENTIATES THE ANTI-PLATELET EFFECTS OF PROSTACYCLIN AND NITRIC OXIDE

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When P2Y12 receptors on platelets are blocked by commonly used anti-platelet drugs such as clopidogrel and prasugrel, the inhibitory brake on adenylate cyclase (AC) activity is lifted and the anti-platelet effects of prostacyclin (PGI2) and other agents that activate platelet AC are synergistically enhanced. We have recently demonstrated that blockade of the P2Y12 receptor also enhances the anti-platelet effect of nitric oxide (NO). The aim of this study was to study the interaction between PGI2, NO and P2Y12 receptor inhibition on platelet aggregation and to determine pharmacologically, using isobolographic analysis, if this interaction constitutes a true synergy or simply an additive effect.

**Methods** Blood was collected by venepuncture into 0.32% trisodium citrate. Platelet rich plasma (PRP) was isolated by centrifugation and either tested directly or further washed with Tyrode’s-Hepes buffer. The PRP or washed platelets (WP) were incubated with the P2Y12 inhibitor prasugrel active metabolite (PAM; 3 μM) or vehicle (0.5% DMSO) for 30 min followed by 1 min incubation with the NO donor DEA/NONOate (10 nM–1 mM) and/or PGI2 (0.2 nM–100 nM) and/or vehicle (0.01M NaOH). WP platelet aggregation to thrombin (1U/ml) was measured by 96-well aggregometry and PRP platelet aggregation to TRAP-6 (Thrombin Receptor Activating Peptide-6, 30 μM) or collagen (50 μg/ml) was measured by light transmission aggregometry (LTA). Isobolograms were constructed by plotting the IC50 values for DEA-NONOate and PGI2 in vehicle or PAM treated WP. Data represents mean±SEM % final platelet aggregation from 4–5 healthy volunteers.

**Results** Thrombin (1U/ml), TRAP-6 (30 μM) or collagen (30 μg/ml) all produced robust aggregation responses in WP and PRP respectively which was largely unaffected by the addition of 10nM DEA-NONOate and 4nM PGI2 or 3 μM PAM. However, the combination of all three (NO, PGI2 and PAM) resulted in almost complete inhibition of platelet aggregation.

Isobolographic analysis of the data showed that the interaction between DEA/NONOate, PGI2 and PAM in WP was strongly synergistic (isoboles curved away from the predicted linear line for an additive relationship).

**Conclusions** These data confirm that activation of platelet P2Y12 receptors by secreted ADP limits the anti-platelet effects of both NO and PGI2, suggesting that P2Y12 activation may be an important mechanism for haemostasis. In addition, we have demonstrated that the interaction between P2Y12 receptors and vascular mediators is strongly synergistic. Potentiation of the effects of endogenous NO and PGI2 may represent an important mechanism for how P2Y12 inhibitors produce anti-thrombotic protection.