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

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When $P2Y_{12}$ 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 (PGI_2) and other agents that activate platelet AC are synergistically enhanced. We have recently demonstrated that blockade of the $P2Y_{12}$ receptor also enhances the anti-platelet effect of nitric oxide (NO). The aim of this study was to study the interaction between PGI_2, NO and $P2Y_{12}$ 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 $P2Y_{12}$ 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 PGI_2 (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 (30 μg/ml) was measured by light transmission aggregometry (LTA). Isobolograms were constructed by plotting the IC_{50} values for DEA-NONOate and PGI_2 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 PGI_2 or 3 μM PAM. However, the combination of all three (NO, PGI_2 and PAM) resulted in almost complete inhibition of platelet aggregation.

Isobolographic analysis of the data showed that the interaction between DEA/NONOate, PGI_2 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 $P2Y_{12}$ receptors by secreted ADP limits the anti-platelet effects of both NO and PGI_2, suggesting that $P2Y_{12}$ activation may be an important mechanism for haemostasis. In addition, we have demonstrated that the interaction between $P2Y_{12}$ receptors and vascular mediators is strongly synergistic. Potentiation of the effects of endogenous NO and PGI_2 may represent an important mechanism for how $P2Y_{12}$ inhibitors produce anti-thrombotic protection.

% aggregation WP	Vehicle	+ 10nM DEA/NONOate and 4nMPGI_2	3μMPAM	3μMPAM + 10nM DEA/NONOate and 4nMPGI_2
1U/ml Thrombin	90±2%	94±2%	78±5%	-5±1%

% aggregation PRP	Vehicle	+ 10nM DEA/NONOate and 4nMPGI_2	3μMPAM	3μMPAM + 10nM DEA/NONOate and 4nMPGI_2
30μM TRAP-6	71±2%	51±2%	67±1%	1±1%
30μg/ml Collagen	65±6%	54±5%	62±5%	14±6%

Figure 1

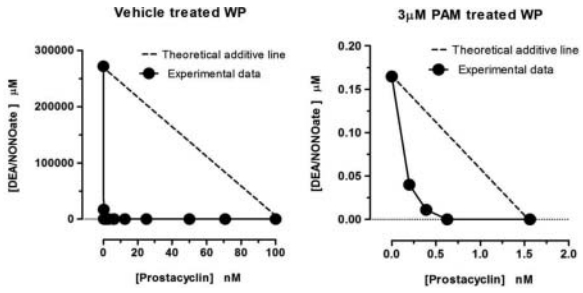

Figure 2