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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 μ M) 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 μ M) or collagen (30 μ g/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 \pm SEM % final platelet aggregation from 4–5 healthy volunteers.

Results Thrombin (1U/ml), TRAP-6 (30 μ M) or collagen (30 μ g/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 μ M 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.

% aggregation WP	Vehicle	+ 10nM DEA/NONOate and 4nMPGI ₂	3 μ MPAM	3 μ MPAM+ 10nM DEA/NONOate and 4nMPGI ₂
1U/ml Thrombin	90 \pm 2%	94 \pm 2%	78 \pm 5%	-5 \pm 1%

% aggregation PRP	Vehicle	+ 10nM DEA/NONOate and 4nMPGI ₂	3 μ MPAM	3 μ MPAM+ 10nM DEA/NONOate and 4nMPGI ₂
30 μ M TRAP-6	71 \pm 2%	51 \pm 2%	67 \pm 1%	1 \pm 1%
30 μ g/ml Collagen	65 \pm 6%	54 \pm 5%	62 \pm 5%	14 \pm 6%

Figure 1

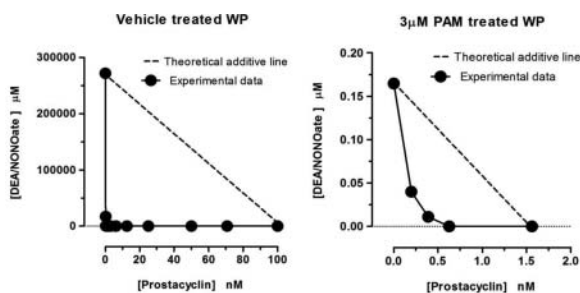


Figure 2