The inhibitory effects of nitric oxide and prostaglandin I₂ on platelet aggregation are greatly enhanced by blockade of P2Y₁₂ receptors

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P2Y₁₂ receptor antagonists such as clopidogrel and prasugrel that block the pro-aggregatory effects of ADP upon platelets are widely used for anti-thrombotic protection. Activation of P2Y₁₂ receptors by ADP leads to inhibition of platelet adenyl cyclase and a reduction in intraplatelet levels of cAMP, resulting in an increase in platelet reactivity. Notably, cAMP synergises with cGMP to produce greater platelet inhibition than cAMP alone, and we have recently shown that P2Y₁₂ receptor activation strongly reduces the platelet inhibitory effects of intraplatelet cGMP (1), in addition to its interaction with cAMP (2). In the normal circulation, endothelial cells produce prostaglandin I₂ (PGI₂) and nitric oxide (NO) which increase intraplatelet levels of cAMP and cGMP and synergise to inhibit platelets. Here we have continued to test our hypothesis that part of the anti-thrombotic effect of P2Y₁₂ receptor antagonists is explained by their ability to potentiate the effects of cAMP and cGMP and so amplify the existing in vivo synergy between PGI₂ and NO.

Blood was obtained by venepuncture from 8 healthy volunteers into 0.32% (w/v) tri-sodium citrate and platelet rich plasma (PRP) was produced by centrifugation. PRP was then incubated with the P2Y₁₂ receptor blocker prasugel-active metabolite (PAM, 1.5, 3 and 6µM; or vehicle, 0.5% DMSO). Light transmission aggregometry was then used to determine the aggregation of PAM-treated platelets in response to ADP 20µM, collagen 4µg/ml or TRAP-6 25µM in the presence of PGI₂ 1nM and/or DEA/NONOate 100nM and/or vehicle. Data was analysed by 2-way ANOVA, and p<0.05 was taken as significant.

Combination of PGI₂ and NO with PAM produced very much greater inhibitions of aggregation than PAM alone. For instance, platelet aggregation in response to TRAP-6 in the presence of PAM 3µM + vehicle was 65±4%, PAM + PGI₂ 63±1%, PAM + DEA/NONOate 55±3%, and PAM + PGI₂ + DEA/NONOate 20±6% (p<0.0001 vs. vehicle). In the presence of PAM 1.5µM, collagen induced platelet aggregation of 74±3% in the presence of vehicle, PAM + PGI₂ 60±10%, PAM + DEA/NONOate 58±6%, and PAM + PGI₂ + DEA/NONOate 21±10% (p<0.0001 vs. vehicle). Similarly, in the presence of PAM 1.5µM, ADP induced platelet aggregation of 70±5% in the presence of vehicle, PAM + PGI₂ 49±13%, PAM + DEA/NONOate 30±7%, PAM + PGI₂ + NO 12±2% (p<0.001).

These studies support our previous finding that blockade of P2Y₁₂ receptors potentiates the anti-aggregatory effects of NO and demonstrate that even partial blockade of P2Y₁₂ receptors in the presence of low concentrations of NO and PGI₂ can produce strong inhibition of platelet aggregation. This may well be relevant to the efficacy of anti-platelet therapies in patients with endothelial dysfunction.

References