Cross regulation of thromboxane A2 (TP) and $P2Y_{12}$ receptors in human platelets

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Introduction: Platelet activation is central to the development of the arterial thrombosis, a major cause of morbidity and mortality in heart disease. Major therapeutic approaches to the treatment of this condition, aspirin and the thienopyridines involve targeting two platelet G protein-coupled receptors (GPCRs) the thromboxane A2 (TP) and $P2Y_{12}$ receptor. A significant number of patients still suffer thrombotic events while undergoing either therapy, termed resistance. Work in our laboratory has demonstrated the existence of heterologous desensitisation between P2Y and TP receptor signalling in human platelets (Barton et al. 2008). In this study we have investigated whether aspirin or thienopyridine (clopidogrel and prasugrel) therapy could lead to 'paradoxical' up-regulation of platelet activation as a consequence of a disruption of P2Y-TP receptor cross-talk.

Method: Experiments were conducted on platelets taken from patients or healthy volunteers following informed consent (Barton et al., 2008). Platelet function was assessed using light transmission or multi-plate impedance aggregometry as previously described (Barton et al., 2008). Receptor surface expression was assessed in human platelets using radioligand binding or in cell lines by ELISA, as previously described. (Mundell et al., 2008)

Results: Increased TP receptor responsiveness was observed in a number of patients following treatment with clopidogrel, where effective $P2Y_{12}$ receptor blockade was achieved. An on-going patient study has also shown a significant number of patients (9/40) have a 30% or greater increase in TP receptor responsiveness following treatment with prasugrel. In a parallel study in healthy volunteers aspirin treatment increased P2Y receptor responsiveness. Given these findings further studies examined the consequences of TP or P2Y receptor stimulation upon receptor expression in human platelets or cell lines. In platelets TP receptor stimulation reduced P2Y receptor surface expression by 50% or more. Cell line studies showed that TP receptor stimulation reduced P2Y receptor surface expression by up to 25% whilst activation of P2Y receptors also promoted TP surface receptor loss of up 25%.

Conclusion: Our results show that inhibiting $P2Y_{12}$ receptors in human platelets can lead to an upregulation of TP receptor signalling. Similarly inhibition of TP receptor activity enhances P2Y receptor signalling. Our studies also show that TP or P2Y receptor activation can promote surface receptor loss P2Y or TP receptors respectively. These findings have significant implications for the therapeutic use of aspirin and the thienopyridines. For example is resistance to thienopyridine therapy in part due to enhanced TP receptor signalling whilst can aspirin treatment lead to enhanced P2Y responsiveness and thus hyper responsiveness to ADP? Further ongoing studies will allow us to demonstrate the significance of our findings and to elucidate the molecular mechanisms responsible.

References:

Barton JF, Hardy AR, Poole AW, Mundell SJ (2008). Reciprocal regulation of platelet responses to P2Y and thromboxane receptor activation. *J Thromb Haemost* **6**(3)**:** 534-543.

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