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Characterising the molecular mechanism of action of the antiplatelet drug ticagrelor (AZD-6140)

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Platelet activation is central to the development of arterial thrombosis, a major cause of morbidity and mortality in heart disease. As an important mediator of haemostasis and thrombosis, ADP influences platelet activation by the coordinated stimulation of $P2Y_1$ and $P2Y_{12}$ receptors ($P2Y_{12}R$). Several $P2Y_{12}R$ antagonists, including ticagrelor are used in the treatment of acute coronary syndromes. The mechanism of action of the drug has not yet been fully characterised. Recent studies have shown that in addition to antagonism of the $P2Y_{12}R$, ticagrelor also prevents platelet aggregation by inhibiting the endonucleoside transporter 1 (ENT1) on red blood cells. This results in an extracellular increase in adenosine levels which stimulates platelet G_s-coupled adenosine receptors, inhibiting platelet activity (1). The aim of our study was to determine whether adenosine uptake inhibition can still occur in the absence of red blood cells and also fully characterize the mode of action of this drug.

We conducted a series of experiments on washed platelets, isolated from whole blood donated by healthy volunteers as previously described (2). The ability of ticagrelor to affect platelet activity was determined using Western blotting analysis of changes in vasodilator-stimulated phosphoprotein (VASP) phosphorylation. Changes in phosphoVASP (pVASP) levels are a sensitive measure of changes in platelet cyclic adenosine monophosphate (cAMP), whereby increased cAMP production or VASP phosphorylation correlates to inhibition of platelet activation (3). Data are expressed as mean±SEM and analysis was performed using one-way ANOVA followed by Bonferroni's post-hoc test where applicable.

Ticagrelor treatment of human platelets induced a time (0-60 min) and concentration-dependent (1 nM - 10 µM) increase in pVASP levels. Ticagrelor treatment (10 µM; 60 min) increased pVASP levels compared to vehicle controls (43.17±11.5% vs 4.32±1.5%; n=5; $p<0.001$). Further investigations revealed that treatment with the selective A_{2A} adenosine receptor antagonist SCH442416 (1 µM; 60 mins) attenuated ticagrelor (10 µM; 60 mins)-stimulated increases in pVASP (69.37±16.3% vs 7.36±2.8%; n=4; $p<0.001$) whilst treatment with the A_{2B}-selective antagonist PSB 603 (1 µM) had no effect. Interestingly further studies revealed that following adenosine receptor blockade, there was still a significant increase in VASP phosphorylation induced by ticagrelor (10 µM; 60 min) treatment compared to the vehicle controls (3.48±0.6% vs 0.95±0.2% increase in pVASP; n=4; $p<0.001$), hinting at the possibility that the drug may also be able to act as an inverse agonist at the Gi-coupled $P2Y_{12}R$ in human platelets.

In summary, we provide evidence that ticagrelor can increase extra-platelet adenosine in the absence of red blood cells, likely by inhibiting a platelet-expressed ENT to activate platelet A_{2A}-adenosine receptors, thereby inhibiting platelet activity. In addition, ticagrelor appears to be able to modulate the basal activity of the $P2Y_{12}R$,

possibly behaving as an inverse agonist at this receptor. Work is currently underway to further confirm the inverse agonistic properties of ticagrelor and quantify possible changes in $P2Y_{12}$ platelet surface receptor expression due to ticagrelor treatment.

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(2) Barton JF *et al*. (2008). *J Thromb Haemost* **6**: 534-543.

(3) Aleil B *et al.* (2005). *J Thromb Haemost* **3**: 85-92.