Potential Off-Target Activity of Proteinase-Activated Receptor Pepducins

G.M. Sloan[1],H. Van De Langemheen[2],G. McConnell[1],R. Plevin[1],R. Liskamp[2],M. Cunningham[1]

1University of Strathclyde
2University of Glasgow

Thrombin-mediated platelet activation and aggregation occurs through a class of G-Protein coupled receptors (GPCRs) called proteinase-activated receptors (PARs). PAR1 and PAR4 are responsible for the thrombin-mediated activity in human platelets that precedes thrombus formation. As such, these have become desirable anti-platelet targets for cardiovascular therapies. Current drugs that target PARs include small molecule competitive PAR1 antagonist Vorapaxar (Chackalamannil et al., 2008). However, the outcome of clinical trials for Vorapaxar revealed an increased risk of intracranial haemorrhage in patients which has limited its clinical use (Morrow et al., 2012). This has led to the development of new ways to target this receptor family.

A class of compounds known as pepducins have been developed to allosterically modulate PAR activity (Covic et al., 2002). PZ-128 is the first example of a pepducin to enter clinical trials as a potential anti-platelet therapy, having passed phase I trials as a PAR1 inhibitor (Gurbel et al., 2015).

As part of a screen for selective PAR pepducins, PZ-128 and the PAR1 competitive antagonist SCH79797 were included for comparison. The allosteric behaviour of PZ-128 was evident in Schild plots (calcium data) which demonstrated a clear deviation away from the line of unity (slope = 0.52 +- 0.13, N = 5) compared to SCH79797 (slope = 1.14 +- 0.16, N = 5). Despite inhibiting PAR1 responses, addition of PZ-128 to HEK293 cells was found to mediate a concentration dependent increase in calcium (LogEC50 = -4.059 +- 0.064, N = 6) and trigger MAP kinase signalling. When compared to the transient PAR1 peptide (TFLLR-NH2) responses, PZ-128 responses were more sustained (>30 mins). Interestingly, PZ-128 activity was similarly observed in mouse platelets, which do not express PAR1 (LogEC50 = -4.339 +- 0.208, N = 6).

These results highlight potential off-target activity of PZ-128 which is currently in clinical trials as a potential anti-platelet therapy. Further work is currently under way to elucidate the mechanism of action of PZ-128 and the physiological consequences of these off-target effects.