**Title (in sentence case):**

Colchicine alters ROS generation in response to GPVI stimulation in platelets

**Aim:**

Colchicine is traditionally used to treat inflammatory conditions e.g., gout, and more recently use has expanded to treat pericarditis and the prevention of heart attack and stroke. It has antiplatelet effects, the mechanisms of which are not well understood. We aimed to determine if colchicine altered platelet activation responses after stimulation of the collagen receptor glycoprotein (GP)VI and the ADP receptor P2Y12 *in vitro*.

**Method:**

Blood from healthy volunteers (n=4-10) was used to examine the effect of typical plasma concentrations of colchicine (20 nM) and higher, microtubule-inhibitory concentrations (2 mM). Platelet responses to stimulation of GPVI and P2Y12 in the presence of *in vitro* colchicine (30 min, 37°C) were examined by Multiplate™ aggregometry (whole blood and platelet rich plasma [PRP]) and flow cytometry (ROS generation and platelet activation markers). Western blots (e.g., phosphotyrosine) were used to further assess the effect of colchicine on the GPVI signalling pathway. Data were analysed with students paired t-test and ANOVA with analysis for trend, p<0.05 was significant, presented as mean±SD.

**Results:**

Colchicine led to a significant decrease in aggregation (whole blood and PRP, 68.1±19.2 v 60.2±16.2 AUC, p=0.006 and 78.0±10.7 v 73.3±12.9 AUC, p=0.005 respectively; vehicle v 20 nM colchicine) in response to collagen stimulation, but ADP stimulation was significant only at 2 mM colchicine. There was a significant decrease in ROS generation (H2DCF-DA) with cross-linked collagen related peptide (CRP-XL) at both colchicine concentrations. Platelet activation marker, CD62P, was inhibited by 2 mM colchicine (both CRP-XL and ADP). Colchicine led to a significant, concentration dependent, decrease in phosphotyrosine (100 kDa) both in response to CRP-XL stimulation (trend, p=0.04) and without stimulation (trend, p=0.03).

Figure: ROS generation in platelets; mean±SEM

**Conclusion:**

Colchicine inhibits collagen-mediated platelet aggregation via the GPVI receptor at physiologically relevant concentrations, inhibits platelet degranulation at higher concentrations, and demonstrates effects on ROS and phosphotyrosine formation.