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Modulation of platelet-monocyte interaction by Annexin A1

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Introduction: There is emerging evidence that platelets have a role in mediating immunity. Previous studies have shown that the glucocorticoid prednisolone inhibits platelet-platelet and platelet-leucocyte interaction[1, 2], however the mechanism of action of glucocorticoids in anucleate platelets remains unexplained. Annexin A1, an endogenous anti-inflammatory protein has been identified as an important mediator for the non-genomic actions of glucocorticoids. We therefore hypothesised that annexin A1 acts as a mediator between glucocorticoids and platelets and so investigated the influences of prednisolone and human recombinant annexin A1 (hr-anxA1) on platelet function.

Method: Human whole blood was obtained from healthy volunteers and was incubated (20mins, 37℃) with prednisolone (0.01, 0.1, 1, 10, 30 µM), hr-anxA1 (3, 10, 30, 100, 300ng/ml) or vehicle (0.1% ETOH or HEPES-NaCl buffer respectively). Samples were then incubated with a range of known platelet activators: arachidonic acid (0.03-1mM), collagen (0.03-30µg/ml) thrombin receptor activating peptide 6 (1-30µM), thromboxane A_2 mimetic U46619 (0.03-30µM), and 2-methylthio-adenosine diphosphate, 2-MESA-ADP (0.03-30µM) and responses assessed by 96-well plate based aggregation with subsequent flow cytometry[3]. Separately, to assess platelet (CD61) - monocyte (CD14) interaction whole blood was either used immediately, or incubated with tumour necrosis factor (TNF-α, 100ng/ml; 4h; 37℃), prior to stimulation with U46619 (0.03 µM) and subsequent analysis by flow cytometry. Platelet P-selectin (CD62P) expression was also measured using flow cytometry. Data is reported as mean±SEM and was analysed using a paired two-way or one-way ANOVA, as appropriate.

Results: Prednisolone (1µM) inhibited platelet aggregation induced by 2-MESA-ADP ($LogEC_{50}$ -6.1±0.3M vs. -5.6±0.3M, n=5-9), but not to any other agonists. Hr-anxA1 did not inhibit any aggregatory responses. Prednisolone (30µM) also inhibited platelet-monocyte interaction in a concentration-dependent manner in normal (63±7% vs. 6±1%, n=4) and in TNFα-treated whole blood (60±2% vs. 20±1%, n=4). This effect was mirrored by hr-anxA1 (300ng/ml) in TNFα-treated whole blood (66±9% vs. 13±5%, n=4). Neither prednisolone nor hr-anxA1 affected platelet P-selectin expression.

Conclusions: Prednisolone inhibited both platelet-platelet and platelet-monocyte interactions. Hr-anxA1 inhibited platelet-monocyte, but not platelet-platelet interactions, particularly in TNFα-treated whole blood, suggesting that annexin A1 may mediate key anti-inflammatory effects. The absence of inhibition by prednisolone or hr-anxA1 of P-selectin expression suggests the involvement of alternative mechanisms and require further study, which could lead to the development of future targeted therapeutics.

References:

1. Liverani E *et al*. (2012). *Biochemical Pharmacology* **83**: 1364-1373.

2. Moraes L *et al*. (2005). *Blood* **106**: 4167-4175.

3. Armstrong P *et al*. (2015). *Blood* **126**: e11-e18.