

Modulation of endothelial behaviour by platelets during acute sterile inflammation

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Severe injuries, such as major trauma, can trigger intense inflammation throughout the body, even when no infection is present, known as “sterile inflammation”. It begins when damaged or dying cells release molecules known as damage-associated molecular patterns (DAMPs) such as histones, and other nuclear and cytosolic proteins such as HMGB1 and S100A8/9, which act as signals that warn the body that tissue has been harmed. Platelets, blood cells traditionally associated with clotting, respond rapidly to these signals. Emerging evidence suggests that platelets could play an active role beyond haemostasis, communicating directly with leukocytes and endothelial cells, the cells that line the inner surface of blood vessels. This platelet-endothelial interaction can change how endothelial cells behave, increasing their stickiness and permeability, and promoting the recruitment of immune cells such as neutrophils from the blood into damaged tissue, which has been shown in diseases such as sepsis. We hypothesize that platelets could act as an intermediate, exacerbating the response of endothelial cells to DAMPs in the context of major trauma, through becoming procoagulant and releasing small molecules. While this response is important for clearing debris and initiating repair, an excessive or dysregulated platelet-endothelial interaction could lead to widespread vascular damage, contributing to complications such as organ dysfunction and acute respiratory distress syndrome (ARDS). Therefore, this is an important therapeutic avenue to explore further.

The aim of the project was to define how platelets influence endothelial cell activity following exposure to DAMPs. To address this aim, we initially characterised platelet responses to DAMPs. Blood was collected from healthy volunteers and platelets were isolated ($3 \times 10^8/\text{mL}$) and recalcified. They were then incubated with S100A8/9 (0-40 $\mu\text{g}/\text{mL}$), Calf Thymus Histones (0-100 $\mu\text{g}/\text{mL}$), HMGB1 (0-10 $\mu\text{g}/\text{mL}$) or CRP-A (3 and 10 $\mu\text{g}/\text{mL}$) and Thrombin (1U/mL) at 200rpm at 37°C for 30 minutes. Platelets were then stained with CD61-APC, CD62-BV421, and Annexin V-FITC for 30 minutes at room temperature. Samples were diluted in AnnexinV binding buffer and analysed on the NovoCyte 3000 flow cytometer, recording 10,000 CD61⁺ events.

The second part of the experiment focused on the co-incubation of platelets and endothelial



cells. HUVEC (Human Umbilical Vein Endothelial Cells) P2-5 were cultured on 0.1% gelatine in 96-well plates, seeded at 30,000 cells/well. The next day they were treated with calf thymus histones (50 µg/ml) with or without washed platelets (100:1, 2 hours, 200rpm, 37°C). They were then detached with accutase, stained with antibodies (VWF-AF555, CD62P-BV421, CD106-APC, CD31-BV785) and then run on the NovoCyte 3000 Flow Cytometer, with the same acquisition described previously.

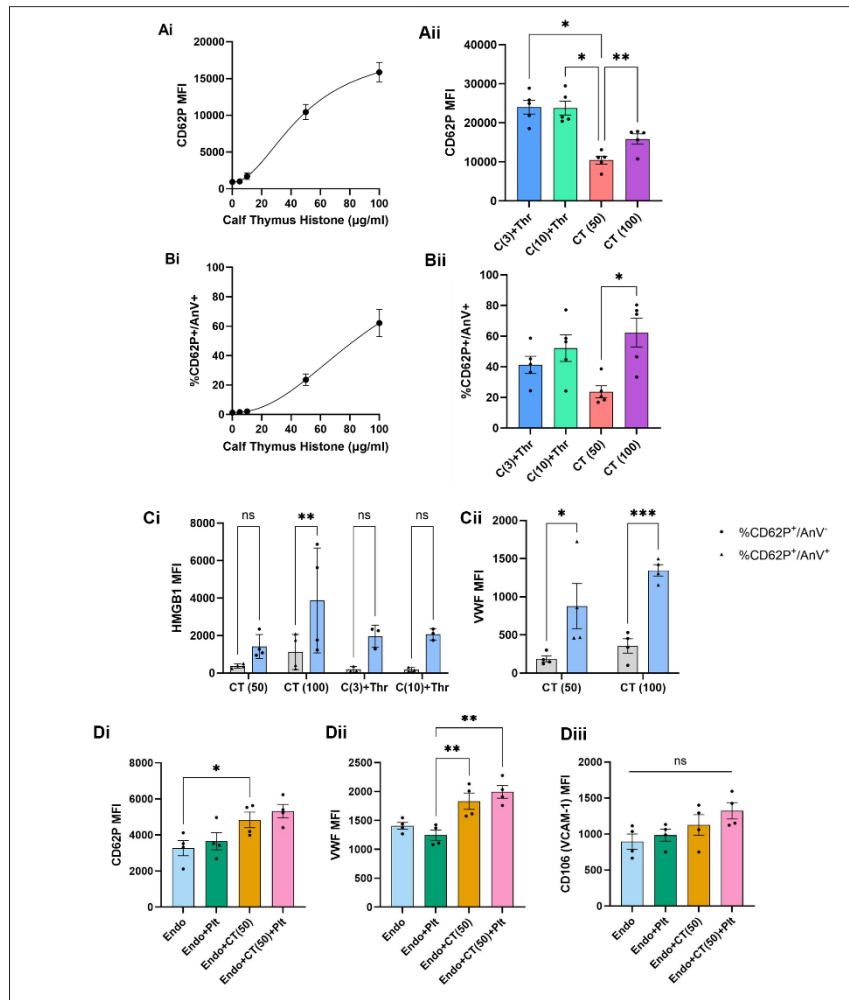


Figure 1: Modulation of platelet and endothelial cell behaviour using DAMPs

Concentration-response of platelets to different DAMPs as quantified by **A)** MFI of CD62P **B)** % CD62P⁺/AnnexinV⁺ **i)** Calf Thymus Histones (0-100µg/ml) **ii)** CRP-A (3 and 10µg/ml) + Thrombin (1U/ml) and Calf Thymus Histones (50 and 100µg/ml). **C)** Differences in surface receptor expression on procoagulant (CD62P⁺/AnnexinV⁺) and degranulated platelets (CD62P⁺/AnnexinV⁻) **i)** MFI of HMGB1 **ii)** MFI of VWF. **D)** Expression levels of endothelial surface receptors, incubated with platelets (Plt), calf thymus histones (CT 50µg/ml) and platelets + calf thymus histones (CT+Plt) **i)** CD62P **ii)** VWF **iii)** CD106. Analysed using non-linear curve fit (Ai, Bi), One-Way ANOVA (Aii, Bii, Di-iii) and Two-way ANOVA (Ci and ii), * p<0.05 ** p<0.01 *** p<0.001, n=3/4.

Initial characterisation of the DAMPs revealed distinct, differing responses. While HMGB1 (0–10µg/mL) and S100 proteins (0–40µg/mL) failed to induce surface CD62P (P-selectin) expression or phosphatidylserine (PS) exposure above baseline (data not shown), calf thymus (CT) histones drove a strong, dose-dependent increase in both activation markers. At 100µg/mL, CT histones elevated CD62P median fluorescence intensity (MFI) to approximately 16,000 (**Figure 1Ai**) and converted nearly 60% of platelets into a fully procoagulant state (defined as CD62P⁺/Annexin V⁺ **Figure 1Bi**). High-dose CT histones (100µg/mL) induced procoagulant platelet proportions comparable to, and slightly exceeding, conventional dual agonists CRP-A and Thrombin (**Figure 1Bii**). Furthermore, procoagulant platelets exhibited significantly higher surface expression of HMGB1 on their surface, which is released from the cytosol of platelets (P<0.01), as well as VWF (P<0.05) which is released from α-granules, compared to degranulated (CD62P⁺/Annexin V⁻) platelets (**Figure 1Ci and ii**). In endothelial assays using HUVECs, CT histones directly stimulated rapid activation, marked by a significant increase in surface CD62P expression through Weibel-Palade body exocytosis (**Figure 1Di**). While platelets alone did not alter baseline VWF levels on endothelial cells, co-incubation of CT histones with platelets generated a significant synergistic increase in endothelial-bound VWF MFI (P<0.01 compared to control and platelets alone, **Figure 1Dii**). Surface expression of CD106 (VCAM-1) showed an upward trend following combined histone and platelet exposure, though this did not achieve statistical significance within the 2-hour timeframe (**Figure 1Diii**). Overall, these results demonstrate that extracellular histones directly trigger platelet procoagulant transformation, with procoagulant platelets expressing more HMGB1 and VWF on their surface. Trends in the expression of endothelial activation markers suggest that platelets may modulate responses to DAMPs, however this requires further testing.

Working in a laboratory environment gave me the opportunity to develop key practical skills, such as pipetting, serial dilutions, methods such as washing platelets, as well as gaining confidence using equipment such as a centrifuge and flow cytometer. Being involved in day-to-day laboratory work, alongside attending meetings and speaking with other professionals, gave me a much greater appreciation for the precision, attention to detail, and teamwork required to produce reliable scientific results. Above all, I learnt that it is a great privilege to learn from individuals who are so incredibly knowledgeable, well-versed and passionate in the field of platelets and related areas of research. One of the most valuable lessons I took away is that platelets are far more than clotting cells. They play an essential role in the immune system, inflammation, wound healing, and the body's response to injury. In cases of major trauma, platelet function can be the difference between life and death, highlighting the profound clinical impact of this area of research.

This project has strengthened my motivation to pursue a career that combines clinical medicine with academic and research medicine, including undertaking further study through a master's or PhD. I hope to specialise in pre-hospital and emergency medicine, an area in which trauma sciences and platelet represent forefront, pioneering fields of research. Understanding how platelets influence endothelial function and inflammation following major trauma has shown me how fundamental science can directly influence and inform clinical practice, from improving our understanding of post-traumatic complications to identifying new therapeutic

targets. Ultimately, I hope to build a career that bridges the bench and the bedside, using research to inform and improve the care I am able to provide as a clinician.