Platelets: immune responders against periprosthetic joint infection

Trevor S Lloyd[1], Rene F Chun[2], Alan K. Li, Evelyn Castillo, Jeffrey Brown, Autreen Golzar, John S. Adams[3], Michael Yeaman, Nicholas M Bernthal[4]
[1]UCLA David Geffen School of Medicine, [2]Ucla-Orthopedic Hospital, [3]UCLA Orthopaedic Hospital Research Center, [4]UCLA, Dept. of Orthopaedic Surgery

INTRODUCTION: Periprosthetic joint infection (PJI) is the most feared and devastating complication of total joint arthroplasty (TJA). While platelets are well known for their role in primary hemostasis, recent discoveries have demonstrated that they are at the nexus of host antimicrobial defense. Both preclinical and epidemiologic data confirm that platelet deficiencies lead to increased surgical infections. In the millions of TJA performed annually, platelets are therapeutically modified with anticoagulants, most commonly acetylsalicylic acid (ASA), to prevent venous thromboembolisms without consideration of their impact on platelet immunologic function. Here we demonstrate the antimicrobial role of platelets against both Gram-positive and Gram-negative pathogens in an ex-vivo model. Additionally, we characterize the direct inhibitory effects of salicylic acid (SA), principal metabolite of ASA, on the antimicrobial properties of platelets in an ex-vivo model.

METHODS:

In ex vivo human platelet assays, fresh whole blood was collected via venipuncture in BD vacutainer tubes with 3.2% sodium citrate buffer. Donors did not consume any anti-platelet medications or experience any illness in the previous 14 days preceding blood collection. Upon collection, whole blood was immediately centrifuged at 100 x g for 20 min at room temperature to yield a layer of platelet-rich plasma. The upper 2/3 of this layer was collected, counted, and diluted in MEM. 1 million platelets were added to 96-well plates. For bacterial preparation, strains of bioluminescent methicillin-sensitive S. aureus (Xen36, GPF1), methicillin-resistant S. aureus (AH4807), and E. Coli (Xen14) were prepared and diluted in minimum essential medium before being added into a 96-well plate to yield a final amount of 10,000 CFU/well, resulting in a final platelet:bacterium ratio of 100:1 in each well. The inoculated plate was then placed into an IVIS Spectrum for longitudinal, quantitative tracking of bacterial burden over time, measured as total flux (photons/s) with use of Living Image software.

To assess the effect of SA on the microbicidal function of platelets, SA was dissolved in ethanol at room temperature. Platelets were incubated with SA (25ug/mL or 200ug/mL) or ethanol control for 30 minutes. The platelet-SA suspensions were added into a 96-well plate followed by S. aureus (Xen36) bacteria.Longitudinal, quantitative tracking of bacterial burden was monitored by IVIS Spectrum and measured as total flux (photons/s) with use of Living Image software.

RESULTS: Compared to untreated pathogens, platelets directly suppressed metabolically active, rapidly dividing, methicillin-sensitive (Xen36, GPF1) and methicillin-resistant (AH4807) S. aureus, as well as E. Coli (Xen14), for at least 30 minutes after administration (Figure 1). When platelets were pre-incubated with high (200ug/mL) and low (25ug/mL) levels of SA, treatment with high levels of SA resulted in attenuated suppression of S. aureus (Xen36) (Figure 2). Platelets alone and platelets treated with low levels of SA were significantly different from platelets treated with high-dose SA at 10, 15, and 30 minutes ($p=0.0048$, $p=0.0028$, $p<0.0001$, respectively).

DISCUSSION AND CONCLUSION: Our data from these ex vivo experiments supports the hypothesis that platelets play a critical role in the protective immune response. Notably, platelets have a direct, microbicidal effect against common Gram-positive and Gram-negative pathogens associated with periprosthetic joint infection. Our findings also link a dose-dependent impairment of platelet antimicrobial action by the active metabolite of aspirin, salicylic acid. Lower doses of salicylic acid appear to preserve the antimicrobial action of platelets. Ultimately, this study invites further investigation into the optimization of postoperative aspirin administration to better preserve the antimicrobial efficacy of platelets while sufficiently protecting the patient from venous thromboembolism in the setting of TJA.

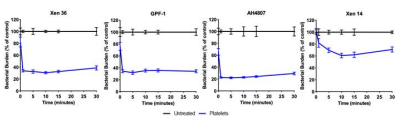

Figure 1: Human platelets directly suppress gram-positive and gram-negative pathogens. At platelet:microbe ratios of 100:1, isolated human platelets rapidly suppress metabolically active, rapidly dividing methicillin-susceptible (Xen36, GPF-1) and methicillin-resistant *S. aureus* (AH4807), as well as *E. coli* (Xen14). Bacterial burden (y-axis) is reported as percentage bioluminescence of untreated controls. Each data point is the mean+SD. In all panels, after 1 minute, untreated bacteria vs bacteria in the presence of platelets were significantly different through 30 minutes ($p<0.001$).

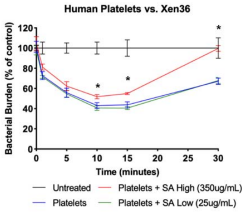

Figure 2: Platelet dysfunction impacts bacterial burden. Isolated human platelets were treated with low vs. high doses of salicylic acid (SA) prior to suspension with Xen36. Treatment with high concentrations of SA resulted in attenuated suppression of *S. aureus* Xen36. At 10, 15, and 30 minutes, MSSA Xen36 in the presence of platelets alone and platelets treated with low dose SA were significantly different from platelets treated with high dose SA ($p=0.0048$, $p=0.0028$, $p<0.0001$, respectively).