Can the use of bacteriophage-derived endolysin prevent and treat *Staphylococcus aureus* periprosthetic infection in a murine model?

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INTRODUCTION: Periprosthetic joint infection (PJI) is a devastating complication following arthroplasty, often necessitating prolonged antibiotics, and staged revision surgery. Despite the development of multi-modal preventative strategies, there has been little to no improvement in clinical outcomes, and breakthrough infections still occur. Hence, there is a need to develop novel antimicrobials. Bacteriophage-derived endolysins are a potential candidate for PJI prevention and treatment. Endolysins have multiple advantages over antibiotics as they do not disturb host flora, can kill antibiotic-resistant bacteria, and rarely induce bacterial resistance. Here, we test the ability of an M23-family endolysin (M23) by itself as well as in conjunction with an anti-biofilm depolymerase, poly-N-acetylglucosamine (DA7), shown in previous in vitro studies to act synergistically with M23 against *Staphylococcus. aureus*, to prevent and treat *S. aureus* PJI in a murine model of post-arthroplasty infection.

METHODS:

To investigate preventative efficacy, we utilized an established PJI mouse model. A 6 x 0.8mm titanium Kirschner wire was implanted retrograde into the distal femur of a 12-week-old C57BL/6 mouse and inoculated with 1000 colony-forming units (CFUs) of bioluminescent *S. aureus* (Xen36) or sterile saline. In the immediate perioperative period, mice were treated with intra-articular sterile saline, intrawound vancomycin powder (2.5mg bolus), or intra-articular M23 endolysin (20mg/kg). Mice received subsequent once-daily injections of either sterile saline or M23 endolysin for 4 days. Bacterial burden was longitudinally measured in vivo by detecting bacterial bioluminescence using an IVIS Spectrum. Animals were sacrificed on postoperative day (POD) 14 to collect implants and harvest tissue for colony-forming unit (CFU) analyses.

To investigate therapeutic efficacy against an established S. aureus biofilm infection, we utilized an established PJI mouse model. A 6 x 0.8mm titanium Kirschner wire was implanted retrograde into the distal femur of a 12-week-old C57BL/6 mouse and inoculated with 1000 CFUs of bioluminescent Xen36 or sterile saline. Mice were separated into different treatment start points (POD3 or POD7). According to group, mice were administered once-daily intra-articular injections of sterile saline, M23 endolysin (20mg/kg), DA7 polymerase (200ug/kg), or M23 endolysin (20mg/kg) and DA7 depolymerase (200ug/kg) for five days. Bacterial burden was longitudinally measured in vivo by quantifying bacterial bioluminescence using an IVIS Spectrum. Animals were sacrificed on POD18 to collect implants and harvest tissue for CFU analysis.

RESULTS:

Compared with infected control implant CFUs, M23 endolysin prevented infection in all animals (p=0.194)(Figure 1). Compared with infected control tissue CFUs, M23 prevented infection in all animals (p=0.005)(Figure 1). In the setting of an established biofilm infection, M23 alone, and when augmented with DA7 co-treatment, did not significantly decrease tissue or implant CFUs(Figure 3).

DISCUSSION AND CONCLUSION:

A *S. aureus*-specific endolysin, M23, both by itself and augmented with an anti-biofilm depolymerase, DA7, was tested in a validated murine model of post-arthroplasty infection. M23 endolysin successfully prevented infection in all three animals. M23 and similar endolysins could be helpful in conjunction with current multimodal strategies to help prevent PJI caused by microbial contamination at the time of surgery. While M23 and DA7 were not shown to be effective in treating an established PJI in this study, further directions could include altering delivery timing, adjusting dosing ratios, or augmenting with antibiotics.

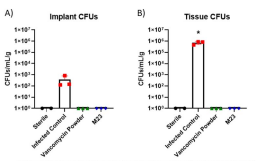

Figure 1: Total colony-forming units from implant samples (A) or tissue samples (B) taken after termination of mice inoculated with *S. aureus* and treated with either intrawound vancomycin powder or intra-articular M23 injections. M23 prevented infection in all animals in both implant and tissue (P=0.194, P<0.005, respectively).

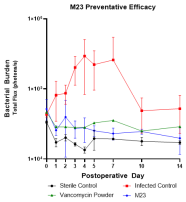

Figure 2: *In vivo* bacterial bioluminescent signal (total flux photons/s) measured throughout course of study.

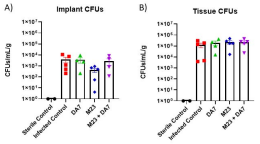

Figure 3: Total colony-forming units from implant samples (A) or tissue samples (B) taken after termination of mice inoculated with *S. aureus* and treated with either intra-articular M23, DA7, or both.

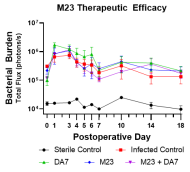

Figure 4: *In vivo* bacterial bioluminescent signal (total flux photons/s) measured throughout course of study.