A Novel Murine Model of Established Staphylococcal Bone Infection in the Presence of a Fracture Fixation Plate to Study Therapies Utilizing Antibiotic-laden Spacers After Revision Surgery

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Introduction: Implant-related bone infections (osteomyelitis) occur in approximately 5% of the 2 million fracture fixation cases annually in the United States [1]. Despite the relatively low incidence, these infections are extremely challenging and costly to treat and often result in high morbidity since treatment requires long courses of systemic antibiotics as well as revision surgeries that involve extensive debridement of the bone and soft tissue. Antibiotic-laden poly-methyl methacrylate (PMMA) is placed into the debridement site to manage dead space and augment the systemic antibiotics with high local doses. PMMA is not biodegradable, however, which results in sequestration of most of the antibiotics. It also requires a second surgery to remove it. Therefore, significant research efforts are focused on exploring alternative biomaterials for local antibiotic delivery. The limited studies that have evaluated local antibiotic delivery in vivo have tested scenarios of prophylactic intervention rather than treatment of an established infection, utilized bone implants that are irrelevant to fracture stabilization, or used larger animals that are not conducive for longitudinal evaluation of the infection and treatments. We hypothesized that a mouse model with an established bone infection in association with a fracture fixation plate, which can accommodate an antibiotic spacer after infected tissue debridement, could recapitulate the salient features of human implant-associated osteomyelitis. Further, this mouse model would enable distinction between significantly different antibiotic therapies through both longitudinal and end-point quantification of the infection and bone.

Methods: All animals were cared for in compliance with regulations of the University Committee on Animal Research. The goal of the first study was to determine the ideal time point for revision following infection. A radiolucent PEEK fixation plate with a 40 nm titanium coating (RISystem, Davos, Switzerland) was installed on the right femur of each BALB/cJ mouse. A 0.7 mm osteotomy was cut in the mid-diaphysis and a collagen sheet, which was either sterile (n=3) or inoculated with 8.0 ± 2.9 x 10^4 colony forming units (CFU) of bioluminescent Staph. aureus (Xen36; n=5), was placed into the defect. The bacterial infection was monitored over 14 days by bioluminescent imaging (BLI), while osteolysis and reactive bone formation were assessed by planar x-ray. At the end of the 14 days, the plates and screws were removed and imaged by scanning electron microscopy (SEM) and the bones were imaged by micro-computed tomography (micro-CT) before preparation for decalcified histology. In the second study, we examined the response of this mouse model to local and systemic vancomycin treatment after the revision surgery. PMMA bone cement was prepared according to the manufacturer’s instructions as a placebo (PBO-PMMA) or mixed with 5 wt% vancomycin (Vanco-PMMA) and formed in a custom mold. After the inoculation surgery as described above, the infection was allowed to establish for 7 days before the revision surgery. During revision, the infected tissue was debrided and the osteotomy was widened to 3 mm to place a PMMA spacer. Select groups also received...
systemic vancomycin beginning at the revision surgery (110 mg/kg twice daily subcutaneously (SC Vanco)). The sample size was at least 8 per group, except for the placebo group that did not receive antibiotics (PBO-PMMA, n=4) due to ethical and animal health concerns. The infection was monitored for an additional 21 days by BLI. The mice were scanned by micro-CT after the revision surgery and at the end of the study to directly measure volumetric changes to the bone. The bone, soft tissue, fixation hardware, and antibiotic implants were separated to count the number of bacterial CFU by homogenization (tissue) or sonication (hardware and implants) and plating dilutions of the suspensions. PBO-PMMA was compared against Vanco-PMMA, with or without SC Vanco, in a two-way ANOVA using Sidak’s test for multiple comparisons. Correlations were examined using the nonparametric Spearman correlation.

**Results:** The active bacterial infection, as measured by BLI, increases to a peak at 5 days post-inoculation and then declines through day 14. Substantial osteolysis began to develop, particularly around the screws, by day 10 post-inoculation. Bacterial colonies and biofilm formation were found on both the plates and screws using SEM after 14 days (Fig. 1a). Gram stains of the histological bone sections revealed bacterial colonies embedded between mature bone and new reactive bone (Fig. 1b), while tartrate-resistant acid phosphatase (TRAP) stains confirmed active bone resorption around the screws. Based on these results, day 7 post-inoculation was chosen as the ideal time point to perform the revision surgery based on the infection peaking by day 5 and the manageable levels of osteolysis that have occurred by this time point.

At the time of the revision surgery, the skin surface was benign but substantial subcutaneous and peri-implant abscesses had formed. The mice did not lose body weight due to the infection over the full 28 days, which suggests that it remained contained as a surgical site infection. The bacterial burden (Fig. 2) and volumetric bone resorption (data not shown) were each significantly reduced by vancomycin administration; however, the effect of Vanco-PMMA was only significant in mice that did not receive SC Vanco. The BLI values at early time points after revision (days 8-12) were significantly correlated with the resorbed bone volume (r=0.43, p=0.009) and total CFU counts (r=0.49, p=0.004) at the end of the study. The bacterial CFU in the bone was also significantly correlated with the total bone resorption (r=0.41, p=0.019). Stability of the plate fixation was maintained in all mice that received vancomycin therapy.

**Discussion:** Implementing an interlocking fracture fixation plate was a critical step to enabling this mouse model of implant-associated osteomyelitis, which can support a revision surgery with extensive tissue debridement and placement of an antibiotic-laden spacer. The value of this infection model is that it presents with many of the important features that make treatment of implant-associated osteomyelitis extremely challenging, including biofilm formation on the implants and septic loosening of the orthopaedic hardware when not properly treated with antibiotics. This study reaffirms the limited benefits of antibiotic delivery via PMMA and these data provide an important baseline for comparison with new local antibiotic therapies in future studies.

**Significance:** This study establishes a novel mouse model of implant-associated osteomyelitis with an interlocking fracture fixation plate, which is appropriate for studying the infection response to both local and systemic antibiotic therapies and allows for revision interventions. Using the mouse as a model readily enabled longitudinal quantification of the infection management through BLI, which was significantly correlated with important outcomes including the osteolytic bone volume and the
persistence of viable bacteria. This mouse model will be an invaluable tool in the assessment of novel biomaterial spacers for local antibiotic delivery and may provide new insights to the biofilm-related disease mechanisms of implant-associated osteomyelitis.

Figure 1. a) Scanning electron micrograph of bacterial colonies with biofilm formation on the fixation plate. Scale bar is 2 μm. b) Gram stained section of the cortical bone demonstrates bacterial colonies (black arrows) embedded within the new bone. c) Alcian Blue/Hematoxylin/Orange G stained section demonstrating the regions of mature bone compared to new bone and the empty osteocyte lacunae (black arrows) within the mature, necrotic bone. White arrows in (b) and (c) indicate the division of old bone and new reactive bone. Scale bars are 200 μm.

Figure 2. a) In vivo BLI demonstrates significant reductions in bacterial load by SC Vanco treatment (*p < 0.05) and by Vanco-PMMA without SC Vanco (**p < 0.05 for PBO-PMMA vs. Vanco-PMMA). Systemic vancomycin treatment significantly reduces bacterial CFU counts in the bone (b) and soft tissue (c). The bacterial CFU counts tend to be reduced with Vanco-PMMA in the mice that did not receive systemic vancomycin, but this effect was not statistically significant. *p < 0.05 for the effect of SC Vanco.

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