Characterization of a Transtibial Amputation Model of Osseointegrated Implant Infection

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INTRODUCTION: Traditional socket-based prostheses for extremity amputations are associated with a high rate of complications and deficits in function, return-to-work/duty, inability to participate in recreational activities, and overall quality of life. The field of osseointegrated (OI) prosthetic implants offers substantial promise for amputee patients, but susceptibility to infection remains a major area of concern. Medical management of these infections has advanced remarkably in recent years, but remains a critical unmet need because of the complexity of the injuries, staged treatment and OI implant placement, and the prevalence of multidrug-resistant organisms. Traditional approaches to treat infections, such as systemic antibiotics and irrigation and debridement procedures, are unable to achieve local efficacy against established infections. To address this critical problem from a research standpoint, a preclinical animal model must exist to fully recapitulate the human model to test potential interventions. Here, we describe a novel rabbit model of OI implant-related infection that can act as a platform for rapid translation and development of therapeutic approaches to combat these uniquely challenging infections.

METHODS: The animal protocol (#A21-042) for this study was approved by the Atrium Health Wake Forest IACUC. Pilot studies (n = 8) were conducted to develop a rabbit transtibial amputation model that mimics the bone-skin-implant interface with an osseointegrated titanium implant. A single-stage transtibial amputation was performed under anesthesia via exposure, transection, reaming, and tapping of the tibia, followed by placement of a 3.5 mm diameter x 75 mm long Ti-6Al-4V DePuy cortical screw implant. The remaining muscle was built into a platform around the implant followed by skin closure and attachment of the prosthetic to the screw. Healing was tracked by comprehensive CBC hematology and clinical chemistry, while osseointegration was observed by radiographic and CT imaging out to 56 days post-op (Fig. 1A). Ambulation and behavior was observed daily. At the terminal endpoint, limbs were examined via high-resolution µCT imaging and histology (Fig. 1B). Separately, pharmacokinetic testing (n = 6) of intraosseous (IO) vancomycin delivery was performed and compared to intravenous (IV) vancomycin delivery as a prelude to investigating the efficacy of antibiotic delivery methods in an infected OI implant model. Samples for pharmacokinetic testing were collected during a non-survival procedure in which the tibia was surgically exposed, and a cortical window was drilled to expose the intramedullary canal. The cortical window was covered, and 30 mg/kg vancomycin in 5 mL was delivered gradually over 30 min via peripheral intravenous cannula in an ear vein (IV) or intraosseous administration via 18G needle (IO). Serum and bone marrow collection was performed prior to vancomycin delivery, at the end of the 30 min delivery period, and once hourly measured from the beginning of the delivery period for 5 hours total. Statistical analysis (2-way ANOVA) was performed in GraphPad Prism 10 software.

RESULTS: Outcomes of the pilot surgeries (n = 8) demonstrated that the rabbits tolerated the surgery and recovery well, and hematology and clinical chemistry results indicate the health of the rabbits is consistently within an expected range over the course of the study. Multiple pilot animals allowed improvement of surgical techniques, prosthetic design, and post-operative care over the course of the studies. Aseptic loosening of the implant was observed in three rabbits (38%), likely due to anatomical features of the rabbit tibia and the immediate weightbearing status needed for quadrupedal animals. For those cases without aseptic loosening of the implant, the final µCT and histology results demonstrate osseointegration between the threads of the implanted screw within the medullary cavity. Pharmacokinetic data further determined that intraosseous vancomycin delivery results in significantly lower vancomycin concentrations systemically as compared to intravenous delivery of vancomycin ($n = 6$, $p < 0.05$ by 2-way ANOVA, Fig. 2A) and a higher peak vancomycin concentration within the tibial canal during the first hour (Fig. 2B).

DISCUSSION: This work-in-progress translational model is poised for the next stage of development. Trials to establish a deep bone implant-associated infection are currently ongoing, and assessment will include CBC hematology and clinical chemistry, radiographic and CT imaging, quantitative microbiology to determine bacterial load, and histology. Preclinical testing of the efficacy of IO administration of vancomycin compared to traditional IV vancomycin delivery will be investigated, with potential for rapid translation to clinical studies.

SIGNIFICANCE/CLINICAL RELEVANCE: Osseointegrated (OI) prostheses have substantial advantages to traditional socket-based prostheses, yet a difficult barrier to its widespread implementation is infection and associated challenges with treating OI-associated infections. The development of a small animal model of intraosseous delivery of vancomycin for OI implant-associated infection will ultimately provide evidence for a clinical trial.

IMAGES AND TABLES:

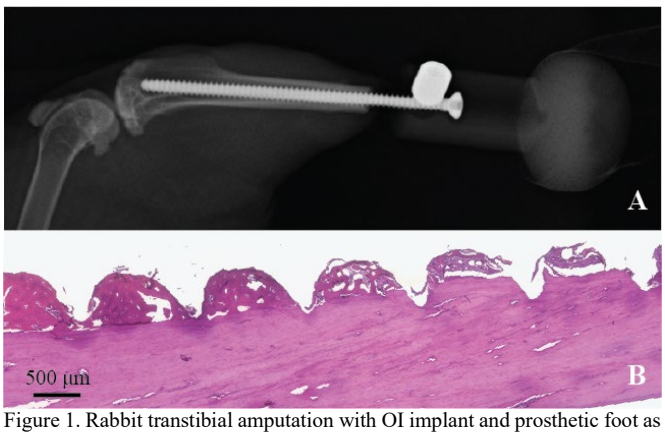

Figure 1. Rabbit transtibial amputation with OI implant and prosthetic foot as seen via CT imaging (A). H&E staining shows bone growth/remodeling in between the threads of the implant (B). Scale bar = 500 µm.

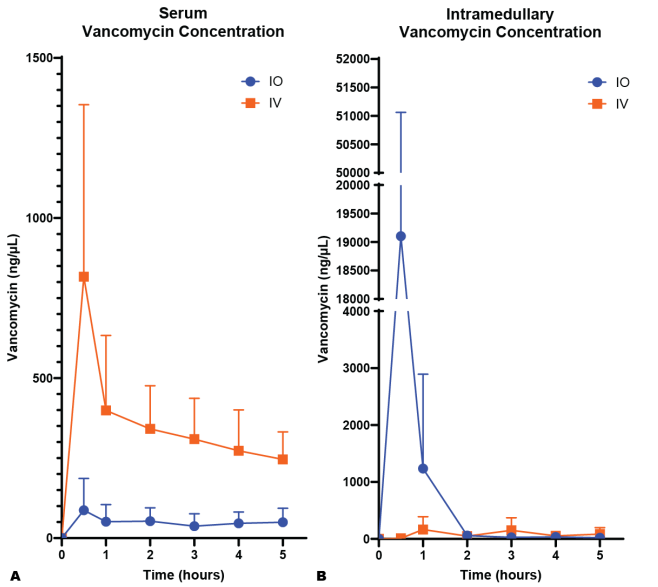

Figure 2. Vancomycin pharmacokinetics comparing intravenous (IV) and intraosseous (IO) delivery. IO delivery resulted in significantly lower serum vancomycin concentration (2-way ANOVA, $n = 6$, $p < 0.05$) (A). The intramedullary concentration of vancomycin was more variable and not statistically different between delivery methods, but the mean peak concentration for IO was higher within the first hour after delivery (B).