A novel rat model of foreign body osteomyelitis for evaluation of antimicrobial efficacy

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ABSTRACT

The most common organism-type causing orthopedic foreign body infection is the staphylococci, of which Staphylococcus aureus and Staphylococcus epidermidis are especially common. These organisms form biofilms on orthopedic foreign body surfaces, rendering such infections challenging and time consuming to treat. Our group evaluates novel therapeutics for orthopedic foreign body infection in animal models. A current limitation of most animal models is that they only allow for the removal of one sample per animal, at the time of sacrifice. Herein, we describe a novel rat model of foreign body osteomyelitis that allows removal of foreign bodies at different time points, from the same infected animal. We demonstrate that this model can be used for both S. aureus and S. epidermidis orthopedic foreign body infection, with 3.56, 3.60 and 5.51 log₁₀ cfu/cm² S. aureus recovered at four, five and six weeks, respectively, after infection, and 2.08, 2.17 and 2.62 log₁₀ cfu/cm² S. epidermidis recovered at four, five and six weeks, respectively, after infection. We evaluated the model with S. aureus infection treated with rifampin 25 mg/kg twice daily for 21 days. Using quantitative cultures, we were no longer able to detect bacteria as of the 14th day of treatment with bacteria becoming detectable again 7 days following the discontinuation of rifampin a period. This novel model allows monitoring of evolution of infection at the infection site in the same animal.

Keywords: Novel model, foreign-body osteomyelitis, rifampin, antimicrobial efficacy.
INTRODUCTION

Joint replacement surgery is increasingly performed as a result of increasing life expectancy, improvements in surgical techniques, and the increased rates of obesity. It was projected that in 2015 there would be almost 400,000 hip replacements and almost 930,000 knee replacements performed in the United States (Kurtz, Ong et al. 2014). These numbers are expected to increase to over 510,000 and over 1.3 million replacements of hips and knees, respectively, by 2020 (Kurtz, Ong et al. 2014). Unfortunately, the rate of prosthetic joint infection (PJI) has remained steady throughout the years, at roughly 1.4-3.3%, depending on the joint being replaced (Tande and Patel 2014). A majority of PJIs are caused by staphylococcal organisms (Staphylococcus aureus and Staphylococcus epidermidis) (Zimmerli, Trampuz et al. 2004, Tande and Patel 2014). The materials that comprise prosthetic joints are conducive to the formation of bacterial biofilms which further complicate treatment. Bacterial biofilms are surrounded by proteins, extracellular DNA and polysaccharides which form a protective polymeric matrix, and are associated with decreased antimicrobial susceptibility by 10-1000-fold when compared to planktonic counterparts (Zimmerli, Trampuz et al. 2004, Esposito and Leone 2008). Bacteria in biofilms grow slowly, rendering growth dependent antimicrobials particularly ineffective; further, implant sites are often poorly vascularized which decreases the ability of antimicrobial agents to reach the site of biofilms, while also impeding host defenses (Zimmerli, Trampuz et al. 2004, Esposito and Leone 2008).

Treatment of PJI can be time-consuming, expensive and arduous. Treatment may involve debridement, antibiotics and implant retention (DAIR) (Esposito and Leone 2008). Unfortunately, there is a 10-20% rate of treatment failure (Berbari and Baddour). The antimicrobial agent rifampin is currently a cornerstone drug in the treatment of PJI caused by staphylococci managed with a DAIR strategy because of its activity against slow-growing staphylococci in biofilms (Zimmerli, Widmer et al. 1998, Zimmerli, Trampuz et al. 2004, Zimmerli 2006). However, rifampin resistance is easily selected when this agent is used alone, with resistance caused by one of several single base pair mutations in the β-subunit of the RNA polymerase (Aubry-Damon, Soussy et al. 1998). Therefore, rifampin is always given in combination with another antimicrobial agent.

We previously observed the appearance and subsequent “disappearance” of rifampin resistance following rifampin monotherapy in a rat foreign body osteomyelitis model (Brinkman, Tyner et al. 2015). Additionally, we demonstrated that previously selected rifampin resistance does not affect subsequent treatment with a rifampin-containing regimen (Brinkman, Schmidt-Malan et al. 2017). A current limitation of many animal models is that they only allow for the removal of one sample per animal, at the time of sacrifice. Herein, we describe a novel rat model of foreign body osteomyelitis that allows removal of three foreign bodies at different time points, from the same infected animal. We used this model to evaluate the effects of rifampin monotherapy vis-à-vis infection, treatment, and selection of resistance.

MATERIAL AND METHODS

Microorganisms: IDRL-6169, a methicillin-resistant S. aureus (MRSA) isolate recovered from a patient with a prosthetic hip infection
and IDRL-8883, a methicillin-resistant *S. epidermidis* (MRSE) isolate recovered from a patient with a prosthetic knee infection, were studied.

**Antimicrobial agent:** Lyophilized rifampin for intravenous administration (Fresenius Kabi, Lake Zurich, IL) was obtained from the Mayo Clinic Pharmacy, and suspended in 10 ml sterile water according to the manufacturer’s instructions to make a stock concentration of 60 mg/ml.

**Novel rat model:** The study was approved by the Mayo Clinic Institutional Animal Care and Use Committee. Chronic foreign body osteomyelitis was established in 8-9 week-old male Wistar rats (Envigo, Madison, WI), weighing between 250-275 grams (Fig 1). Animals were anesthetized by administering ketamine (60 mg/kg) and xylazine (6 mg/kg) intramuscularly. The proximal third of the left tibia was surgically exposed and three-1 mm holes were drilled into the medullary cavity in a perpendicular fashion. A 5 mm x 1 mm stainless steel wire (Zimmer, Warsaw, IN), with either preformed *S. aureus* or *S. epidermidis* biofilm, was inserted into each hole, perpendicular to the bone. A small portion (0.5 mm) of the wire protruded from the bone. Biofilms were formed on the wires by incubating them in a 1 McFarland culture of each organism for two hours at 37°C. Wires were secured by the application of dental gypsum around the holes and the muscle was closed with 3-0 vicryl (Ethicon, Inc., Somerville, NJ). The skin was closed using Tissuemend II (VPL, Phoenix, AZ) and surgical clips. Aluspray (Neogen Corporation, Lansing, MI) and Chew Guard (Summit Hill Laboratories, Tinton Falls, NJ) were applied to the wound. Animals were given buprenorphine (slow release, 60 mg/kg) and meloxicam (slow release, 2 mg/kg) subcutaneously for post-surgical pain control. To remove a wire at specific time points, the left tibia was surgically exposed, and one wire was removed using forceps. If necessary, a bone drill was used around the wire to break up the dental gypsum. Following removal of the wire, the wound was closed in the initial surgery. At the time of the third wire removal, animals were sacrificed using CO₂.

**Establishment of the novel model:** Studies were performed using MRSA or MRSE. Following infection for 28 days, one wire was removed from each of three animals at four, five and six weeks following infection. Wires were placed into 1 ml of trypticase soy broth (TSB), vortexed, sonicated and quantitatively cultured.

**Rifampin monotherapy study:** Using the novel model, foreign body osteomyelitis with MRSA was established for 28 days. The animals were then randomized to treatment and control groups, with 32 animals in each group. Animals in the treatment group were given rifampin (25 mg/kg intraperitoneally, every 12 h) for 21 days. One wire was removed from 8 animals in each group at the following time points: One day prior to rifampin monotherapy, after two, five, seven, 10, 14 and 21 days of rifampin monotherapy, and two, five, seven, 10 and 14 days following rifampin monotherapy completion. Wires were vortexed, sonicated and quantitatively cultured to determine bacterial quantities. Cultures were plated on to trypticase soy agar (TSA) plates containing 5% sheep blood as well as onto TSA plates containing 4 µg/ml rifampin. For samples from which no bacteria were recovered using quantitative culture, qualitative culture was performed by incubating wires in 1 ml TSB overnight at 37°C and plating 100 µl of the broth onto both TSA containing 5% sheep blood agar and TSA containing rifampin 4 µg/ml.
RESULTS

**Development of the novel model:** Experiments with both *S. aureus* and *S. epidermidis* demonstrated establishment of foreign body osteomyelitis. No contamination of the wires was observed. Average bacterial quantities of *S. aureus* at four, five- and six-weeks following infection were 3.56, 3.60 and 5.51 log$_{10}$ cfu/cm$^2$, respectively (Fig 2). Average bacterial quantities of *S. epidermidis* at four, five- and six-weeks following infection were 2.08, 2.17 and 2.62 log$_{10}$ cfu/cm$^2$, respectively (Fig 3).

**Rifampin monotherapy study:** MRSA quantities recovered from the wires of control and treated animals are shown in Table 1. Wires removed from control animals over the course of infection showed similar bacterial quantities at all time points studied (Fig 4A). Wires removed from rifampin-treated animals demonstrated that bacteria were detectable until the 14th day of treatment with rifampin, and remained undetectable through quantitative culture until 7 days following discontinuation of rifampin treatment (Fig 4B). Although not detected through quantitative culture, bacteria were recovered using qualitative culture from wires removed from animals both two and five days following treatment completion (Table 1). In previous studies, resistance was found uniformly two days following completion of rifampin treatment. This was not the case in the current study, where rifampin resistance was not detected uniformly two days following completion of rifampin treatment (bacteria recovered from 1 out of 3 wires were resistant to rifampin). All bacteria recovered from that single wire were rifampin-resistant.

### Table 1. Bacterial quantities of *Staphylococcus aureus* IDRL-6169 recovered from control and rifampin monotherapy animals at various time points during and after treatment (n=8 for each time point).

|                  | Control          | Treatment        |
|------------------|-----------------|-----------------|
|                  | Mean log$_{10}$ | Mean log$_{10}$ |
|                  | cfu/cm$^2$      | cfu/cm$^2$      |
|                  | Standard        | Standard        |
|                  | deviation       | deviation       |
| Pre-treatment    | 4.95 ± 1.05     | 4.68 ± 0.38     |
| 2 days of treatment | 4.80 ± 1.05     | 3.88 ± 0.43     |
| 5 days of treatment | 4.83 ± 1.01     | 3.61 ± 0.27     |
| 7 days of treatment | 4.09 ± 1.12     | 2.24 ± 0.77     |
| 10 days of treatment | 3.69 ± 0.96     | 2.75 ± 0.56     |
| 14 days of treatment | 4.95 ± 1.05     | 0.1 ± 0.14      |
| 20 days of treatment | 4.44 ± 0.48     | 0.1 ± 0      |
| 2 days post-treatment | 4.44 ± 0.48     | 0.1 ± 0.21     |
| 5 days post-treatment | 4.17 ± 0.91     | 0.1 ± 0.14     |
| 7 days post-treatment | 4.39 ± 0.47     | 3.56 ± 0.83     |
| 10 days post-treatment | 3.76 ± 0.38     | 3.48 ± 0.65     |
| 14 days post-treatment | 4.53 ± 0.77     | 3.11 ± 0.95     |

cfu, colony forming units
Figure 1. Illustration of the rat model of foreign body osteomyelitis.

Figure 2. Bacterial quantities of *Staphylococcus aureus* IDRL-6169 initially and 4, 5 and 6 weeks following establishment of infection (n=3 for each time point). Error bars depict standard deviations. cfu, colony forming units.
DISCUSSION

Current foreign body osteomyelitis models in rats usually involve only one foreign body per animal, removed at the time of sacrifice; accordingly, they do not offer monitoring of infection in individual animals over time. The model described here allows for removal of multiple foreign bodies, one at a time, from single animals over time. This model is advantageous in monitoring the evolution of bacteria at an infection site, monitoring the efficacy of a new or existing antimicrobial agent over defined treatment periods or monitoring the response of different foreign body materials in the same animal at the same infection site.

A limitation of the model is that bone surrounding the foreign body cannot be readily cultured; only the foreign body is studied. In our previously described model, both bone and wire surrounding the infection site were removed and cultured separately (Vergidis, Schmidt-Malan et al. 2015). Historically, we have found greater bacterial quantities in bone compared with wires. Additionally, the preformed biofilm on the wire may be providing its own niche unaffected by the surrounding tissue; therefore one could argue that the three foreign bodies are independent of one another. If one uses the model to monitor evolution at the infection site, it may be possible that limited genotypic changes are detected because bacteria within the biofilm are protected and isolated from host influences. An example of this is the lack of uniform rifampin resistance observed in bacteria recovered through qualitative culture two days following treatment completion. To address this potential limitation, it may be possible to inject bacteria into the intramedullary cavity of the tibia, as was done in our previously described model, in future experiments. Additionally, the time between surgeries has to be at least 72 hours for animal husbandry purposes, limiting time points that can be assessed.
CONCLUSION

In conclusion, we have described a novel model of foreign body osteomyelitis that allows removal of more than one sample from individual animals at different times. We demonstrated that this model is feasible using...
both S. aureus and S. epidermidis, with these bacteria recoverable as long as six weeks following infection. Additionally, we used this model to evaluate response of S. aureus infection to rifampin therapy over a period of three weeks. Future uses for this model include monitoring efficacy of antimicrobials and evolution of infection sites.

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