CHARACTERIZATION OF A CHRONIC INFECTION IN AN INTERNALLY STABILIZED SEGMENTAL DEFECT IN THE RAT FEMUR

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INTRODUCTION: A model in the rat femur was developed to simulate chronic osteomyelitis in a fracture fixed with hardware, and treated with débridement surgery and antibiotics. A defect of critical size was surgically created, stabilized with a polyacetyl plate and Kirschner wires, and contaminated with bacteria. After a period of time, the defect was surgically débrided and the rat was treated with/without systemic antibiotic. The purpose of this study was to characterize this model by addressing the following questions: Which combinations of initial bacterial inoculum and time to débridement would reliably produce a chronic infection without generating excessive bony lysis that would compromise the defect fixation by the time débridement was performed (Experiment #1)? Based on an inoculum and time to débridement from Experiment #1, what would be the effect of treatment with/without systemic antibiotic on the débrided defect over time (Experiment #2)?

METHODS: This study was approved by our institutional review board for use of animals in research. **Experiment #1**: A 6 mm defect was surgically created and stabilized in one femur in 192 adult Sprague-Dawley rats. Each of 4 groups of 48 rats received 1 of 4 inocula of *S. aureus* (10^3, 10^4, 10^5 or 10^6 CFUs suspended in saline, sensitive to ceftriaxone) mixed with 60 mg of lyophilized collagen, and packed into the defect. Twelve rats in each group were euthanized at 1, 2, 3 and 4 weeks. Quantitative bacteriology (number of recovered CFUs of bacteria/gram of standardized amount of bone) was performed in 6 rats at each time point. In the remaining 6 rats, a radiographic assessment of bone damage (number of locations with evidence of lysis where the Kirschner wires passed through the cortical bone; possible score of 0 to 12) and linear torsional stiffness of the defect fixation (Figure 1) were determined. **Experiment #2**: Based on the findings from Experiment #1, a segmental defect was created, stabilized and contaminated with 10^5 CFUs of *S. aureus* in 96 rats. After 2 weeks, defects in all rats were surgically débrided and new sterile collagen was packed into the defect. Forty-eight rats received ceftriaxone after débridement (50 mg/kg, 1/day × 28 days, S.O.), and 48 rats did not. Twelve rats in each of these 2 groups were euthanized at 2, 4, 8 and 12 weeks after débridement, with quantitative bacteriology performed in 6 femurs, and radiographic bone damage and fixation stiffness assessed in 6 femurs. Data in both experiments were analyzed with a 2-way ANOVA with p<0.05.

RESULTS: **Experiment #1**: The mean recovered CFUs of bacteria for all initial bacterial inocula significantly increased during the first week after surgery, reaching their peak values, and then significantly declined over the next 3 weeks (Figure 2). The recovered bacteria exhibited a slight rebound at 4 weeks with initial inocula of 10^5 and 10^6 CFUs, but continued to decline for inocula of 10^3 and 10^4 CFUs. The mean stiffness of the defect fixation for all initial inocula significantly decreased from 9% to 25% during the first week after surgery, compared with time zero controls (Figure 3). The mean stiffness for 10^3 and 10^5 CFUs continued to decrease over the next 3 weeks, while the stiffness for 10^4 and 10^6 CFUs showed a trend toward control levels at 4 weeks.

**Experiment #2**: In defects of animals that did not receive antibiotic, the mean recovered CFUs of *S. aureus* peaked at 2 weeks after débridement, and then continually declined to their lowest level at 12 weeks (Figure 4). In defects of animals that received antibiotic, the recovered bacteria decreased to their lowest level at 4 weeks during the period of antibiotic administration, but increased between 8 and 12 weeks, peaking at 12 weeks. The mean recovered bacteria from defects without antibiotic at 2 and 4 weeks were significantly greater than from defects with antibiotic, while the mean recovered bacteria from defects with antibiotic at 12 weeks were significantly greater than from defects without antibiotic.

DISCUSSION: The proposed chronically infected segmental defect model was characterized between the time of initial bacterial contamination and subsequent débridement surgery by generating profiles of the number of recovered CFUs of bacteria, radiographic bone damage, and stiffness of the defect fixation, as a function of initial inoculum and time. The different initial bacterial inocula resulted in different levels of bone damage and fixation stiffness. An initial inoculum (10^5 CFUs) and time to débridement (2 weeks) that resulted in a reliable chronically infected defect without excessive bony lysis and loss of fixation stability was found. This will allow future investigations into the treatment of infected fractures using OP-1 or other growth factors, as well as other topics related to the study of chronic infection. The chronic infection model was further characterized by generating the same profiles as a function of time from débridement surgery, and whether or not antibiotic therapy was administered. Surgical débridement plus antibiotics could suppress the infection, but could not thoroughly eliminate the bacteria or infection. There was evidence that the rat’s immune system was able to fight the infection over time (without antibiotic), but not eliminate it. There was also evidence that antibiotic therapy treated the infection during the period it was administered, but that the infection reestablished itself after the period of antibiotic treatment was completed (with hardware in place). Thus, the model had characteristics of a clinically relevant chronic infection.

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