INTRODUCTION: The increasing use of implantable medical devices, both temporary and permanent, combined with the growing number of immunocompromised patients being treated, has lead to a greater number of nosocomial infections. In order to develop strategies to decrease these infections and to develop effective treatments for them, an animal model that reproduces the salient features of the clinical situation is required. The objective of this study was to modify an existing femur fracture model in the rat to one that could be used as a model of osteomyelitis associated with a closed fracture.

METHODS: 30 male Sprague-Dawley rats (250-300-g) were randomly placed into one of 3 groups (Control, S. aureus, S. aureus + ceftriaxone; n=10 per group). A previously described closed fracture model in the rat was combined with inoculation with a Staphylococcus aureus strain that was isolated from an infected total hip arthroplasty. A medial stifle arthroscopy was used to inoculate 50-µl of bacterial suspension, equivalent to total of 10^7 colony forming units (CFU), of S. aureus (S. aureus and S. aureus + ceftriaxone groups) or PBS (Control group) into the medullary canal of the right femur. A 316L SS pin (1.4-mm x 20-mm) was inserted into the medullary canal and a mid-shaft closed fracture of the right femur was created. Rats in the S. aureus + ceftriaxone group received ceftriaxone (50-mg/kg) every 24 hours until euthanasia at 3 weeks after surgery.

Lateral radiographs of the right femur limb were obtained immediately after surgery and at weeks 1, 2 and 3. Two individuals blinded to study group evaluated the radiographs focusing on three regions of interest (ROI) using a published grading system: (1) proximal metaphyseal area; (2) diaphyseal region involving the site of the fracture; and (3) distal metaphyseal area. The highest possible overall score was 47.

At necropsy both femurs from 8 rats from the S. aureus and 7 rats from each of the Control and S. aureus + ceftriaxone groups were used to determine the CFU of S. aureus per femur and pin. The SS pins were aseptically retrieved from the operated femurs prior to snap freezing. After sonication and vortexing microtiter dilutions were used to determine the CFU/pin after growth of 10-fold dilutions on tryptic soy agar (TSA) for 24-hours at 37°C. Each femur was snap frozen, ground to a powder under sterile conditions, suspended in tryptic soy broth (TSB) and diluted in 10-fold microtiter dilutions. Each dilution was plated on TSA plates for 24 hours at 37°C to determine the CFU/femur. Blood cultures and cultures of both the right and left stifle were obtained at the time of euthanasia.

The right femur from 2 rats (n=6) in each group was used for histopathological evaluation. Decalcified sections were stained with H&E and were assigned scores using a previously published grading scheme using the same ROI as were used for the radiographic evaluations.

Table 1: Mean radiographic score (SE) out of 47. * denotes time post-operative where a statistically significant difference was noted across the three groups. Control n=9; S. aureus n=10; S. aureus + ceftriaxone n=9.

RESULTS: One rat in each of the Control and S. aureus + ceftriaxone group had to be euthanized prior to the end of the study due to incisonal dehiscence/self mutilation. No bacteria were recovered from the left femur of any of the rats regardless of the group. Chi-square analysis revealed a significant difference in the CFU/right femur (p<0.0001) and CFU/pin (p=0.0002) across treatment groups. (Figure 1) Blood cultures were negative for all 28 rats. Stifle cultures were positive in 11/19 of the right stifles of rats that were inoculated with S. aureus and 0/19 of the left stifles. The mean ± SE radiographic scores were similar post-operatively, but were significantly different at weeks 1, 2 and 3 (Table 1). The average histology scores were 25, 82, and 43 for the Control, S. aureus, and S. aureus + ceftriaxone groups, primarily reflecting a periostial remodeling response in the Control group, severe inflammation in the S. aureus group, and a reduced severity of inflammation in the S. aureus + ceftriaxone group.

DISCUSSION: While antibiotic therapy in the present study did not eliminate the bacterial infection, significant treatment differences in the CFU/pin and radiographic scores were observed, and similar trends were observed in the CFU/femur and histological data. None of the rats were systemically ill or had positive blood cultures at the time of euthanasia. The use of an intra-medullary pin in this model provides the potential for future studies to examine the effects of biofilm formation. One limitation of this model was the presence of positive bacterial cultures in 57.9% (11/19) of the operated stifles in rats inoculated with S. aureus. This appears to be associated with the surgical technique itself and we feel can be minimized with refinement of the model. This was a pilot study with a small number of rats, which limits the statistical conclusions that can be made. A major advantage of this model of implant-associated osteomyelitis is that it does not require the creation of a bone defect, an open fracture, or the use of a sclerosing agent to enhance infection.

REFERENCES:

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