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A Model for Chronic Osteomyelitis Using Staphylococcus aureus in Goats

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Animal models of osteomyelitis traditionally have been characterized by inherent weaknesses related to animal size, differences in clinical findings compared with humans, aggressive behavior, high complication rate, and high cost. In this experiment, a model of tibial osteomyelitis was established in 28 goats with a very low complication rate and a consistent clinical, radiographic, and histologic disease course. The infecting Staphylococcus aureus organism was isolated in all but five of 28 animals (82%), in which there was no growth of bacteria at 24 and 72 hours. All five of these specimens had histologic evidence of osteomyelitis. Twenty-seven of the 28 animals (96%) had radiographic and histologic evidence of osteomyelitis. Clinical progression of the disease was observed by draining wounds, a postoperative limp that subsided in all goats, and varied periods of anorexia despite an average increase in body weight. There were no complications or mortalities related to the establishment of the animal model. This large animal model will provide a practical method of studying osteomyelitis and comparing treatment protocols.

Chronic osteomyelitis is a recalcitrant condition in which symptoms have been present for longer than 3 months or in which an initial therapeutic regimen has failed. Waldvogel et al distinguished several forms of osteomyelitis including hematogenous, contiguous focus, associated with vascular disease, and chronic.[10] Cierny et al anatomically distinguished several forms of chronic osteomyelitis as medullary, superficial, localized, and diffuse.[1] Although numerous classification and staging systems for osteomyelitis have been proposed, the influence of numerous confounding variables (eg, pathogenic organism, antibiotic sensitivity, and anatomic location of the disease) on these classifications cannot be easily controlled in clinical studies. As a result, animal models have been developed to control these factors. A good animal model must accurately reproduce the disease in question, be available to multiple investigators, be exportable, be large enough for multiple biopsy samples but also fit into most animal facilities, be capable of being handled with routine resources, and survive long enough to be usable.[4] Many animal models have been hampered by size requirements, purchasing costs, care costs, and comparability to the clinical findings in human osteomyelitis. We sought to establish such a model in an animal that would provide a balance of convenience, size, cost, and consistent response to bacterial inoculum.

We proposed that the goat (Capra hircus) would be a reasonable animal model for osteomyelitis. After inoculation of the proximal tibial metaphysis with Staphylococcus aureus, analyses of the symptoms, gross appearance, hematologic features, radiographic progression, microbiologic analysis, and the histologic characteristics of this model were done to confirm its potential utility.

MATERIALS AND METHODS

Twenty-eight goats (Capra hircus), 1–4 years of age (mean, 2 years), and average weight of 34 kg (range, 25–45 kg) were used in accordance with the Institutional Animal Care and Use Committee at the William Beaumont Army Medical Center. The animals, consisting of one male and 27 females, were obtained

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Each author certifies that the institution where the study was conducted approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.
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from a local farm flock and were cared for in accordance with standard animal care protocols. The animals were housed in a large area with access to hay and water ad libitum and daily supplemental goat pellets.

The anesthetics for the radiographic procedures consisted of xylazine (0.5 mg/kg) with the reversal agent yohimbine (0.11 mg/kg). For the surgical procedures, general anesthesia was induced with ketamine (15 mg/kg) and xylazine (0.5 mg/kg). Subsequently, animals were intubated and maintained with inhalational isoflurane. All animals had a fentanyl patch (25 μg/hour), placed approximately 12 hours before surgery with exchange of the patch at 72-hour intervals as needed for pain relief in the postoperative period.

Staphylococcus aureus (S. aureus) isolated from human osteomyelitic bone was obtained from the American Type Culture Collection (ATCC #700260) and maintained in tryptic soy broth. The bacteria were streak-diluted on a brain-heart infusion agar plate and incubated overnight at 37°C. An isolated colony was transferred to a side-arm culture flask containing 50 mL tryptic soy broth. The culture was incubated at 37°C until midlog phase growth was reached, determined spectrophotometrically at 600 nm (Spectronic 20; Bausch & Lomb, Rochester, NY). The midlog suspension was diluted 1:1 with tryptic soy broth containing 20% glycerol and stored at − 70°C until used. Standard growth curves were done to quantitate S. aureus plaque-forming units. Frozen aliquots were thawed rapidly, transferred to flasks containing prewarmed tryptic soy broth, and incubated at 37°C. At 30-minute intervals, optical densities were measured with a spectrophotometer at 600 nm. Aliquots of 100 μL were plated in triplicate on brain-heart infusion agar. After overnight incubation at 37°C, colony counts were done and the colony forming units (CFU) per mL were plotted against optical densities with time. All animals were inoculated with S. aureus grown from the frozen stock, characterized by a standardized growth curve. At surgery, frozen S. aureus vials were thawed rapidly at 37°C, contents transferred to flasks of prewarmed tryptic soy broth, and incubated while shaking at 37°C until midlog phase was reached. Twenty minutes before injection, optical density readings were done. Using this data, numbers of CFU/mL were determined from the standardized growth curves. Appropriate volumes of culture (containing 4×10^9 CFU) were centrifuged at 3000 rpm for 10 minutes at 4°C. Supernatant fluids were removed by aspiration and cell pellets were resuspended in sufficient volumes of cold phosphate buffered saline to achieve a concentration of 4×10^9 CFU/mL. The S. aureus suspensions were transported immediately to the animal research facility, and 1 mL suspension was injected into previously prepared goat tibiae.

Before surgery, food and water were withheld for 24 hours. A 2-cm skin incision was made approximately 2 cm distal to the tibial tuberosity. The periosteum was incised and elevated over an area approximately 2 cm^2. A 3-mm diameter unicortical drill hole was made. Bone wax (Ethicon, Somerville, NJ) was used to seal the drill hole. An angiocatheter was introduced through the bone wax and 1 cc of 5% sodium morrhuate sclerosing agent was injected. Ten minutes later, cefazolin sensitive S. aureus (4×10^9 CFU) suspended in 1 cc brain-heart infusion broth was injected using a separate 18-gauge angiocatheter. The entry hole was sealed again with bone wax, and the wound was closed in one layer with 2-0 Prolene (Ethicon). Animals were given an intravenous dose of cefazolin 1 hour after surgery to prevent fatal sepsis.

The goats were followed up clinically and radiographically for the next 12–16 weeks. Rectal temperatures and appearance of any external wounds or sinuses were recorded daily.

Standard radiographs (anteroposterior and lateral views) of the bilateral tibiae were obtained before inoculation to rule out any possible contralateral limb disorders. Radiographs were obtained of each of the left (inoculated) tibiae at 2, 6, and 12–14 weeks postoperatively. A radiologist, blinded to the inoculations, reviewed all the radiographs looking for evidence of osteomyelitis based on the presence of lucency, periosteal reaction, sclerosis, and sequestra.

Tissue samples for culture and histologic analysis at the study end point were used to confirm the presence of osteomyelitis. Fragments of debrided soft tissue and bone from the site of injection, contents of any abscess cavities, and deep cultures of the medullary cavity were obtained using a culture swab directly plated onto plates with antibiotic disks. The plates were evaluated by a microbiologist at 24 and 72 hours for colony growth. Necrotic bone and intramedullary debris were sent for histologic examination. Bone was placed directly in buffered formalin, decalcified with Kristensen's solution, embedded in paraffin, and stained with hematoxylin and eosin. A pathologist examined all specimens for evidence of acute and chronic osteomyelitis with evaluation for inflammatory cells, osseous destruction, and fibrosis.

RESULTS

Twenty-seven of 28 study animals had radiographic and histologic evidence of osteomyelitis. The average postinoculation temperature of the goats ranged from 101.5°–103.2ºF (normal range, 101°–103.8ºF) with no animals having fatal sepsis develop (Fig 1).

Wounds with drainage at the surgical site developed in 11 of the inoculated goats. A limp, which disappeared in all animals by postoperative Day 72, was observed immediately after inoculation; however, there were no pathologic fractures.

Radiographic progression of the goat tibiae at 2, 6, and 12–14 weeks after inoculation revealed an initial period of soft tissue swelling, lucency, and periosteal reaction followed by an increase in sclerotic changes and sequestrum in later cases (Fig 2). Radiographic changes were consistent and limited to the proximal tibial diaphysis.

The infecting bacterium, S. aureus, was isolated in 23 of 28 goats (82%). There was no growth of bacteria at 24 and 72 hours in the remaining goats. However, histologic analysis of specimen from these five goats revealed acute and chronic osteomyelitis.

Twenty-seven of 28 bone specimen examined by our hospital pathologist showed acute and chronic inflamma-

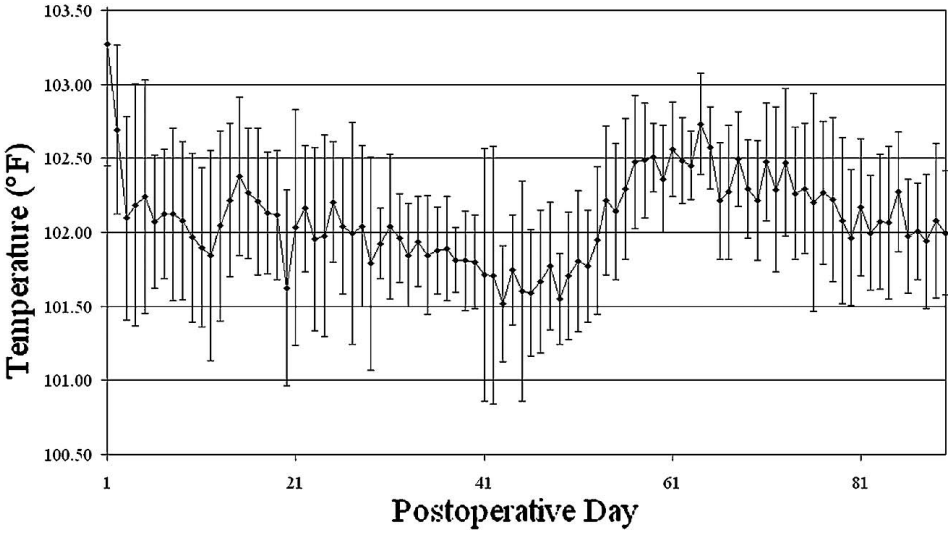

Fig 1. Temperature curves for the goats from the immediate postoperative period to the end point of the study are shown (error bars, ± 1 SD).

tion, osteolysis, and clusters of bacteria with occasional necrotic fragments of bone within areas of fibrosis, all indicators of acute and chronic osteomyelitis (Fig 3).

All animals survived the initial surgery and establishment of osteomyelitis. Two of 28 goats experienced cardiorespiratory arrest and died despite resuscitative efforts shortly after receiving a preoperative sedative (xylazine) in preparation for their second procedure (debridement of tibial wound). These goats were included in the study group (28) because harvest of the tibial bone specimen was possible.

DISCUSSION

We report a model of chronic osteomyelitis in the goat. The value of the model is based on many advantages of the goat over other animals. The goat is large enough to simulate the clinical, radiographic, and histologic characteristics of human osteomyelitis. More importantly, it permits evaluation and comparison of various treatment modalities for this condition. The goat model is associated with a low rate (7%) of mortality, a high degree of radiographic and histologically confirmed osteomyelitis (96%), and a high

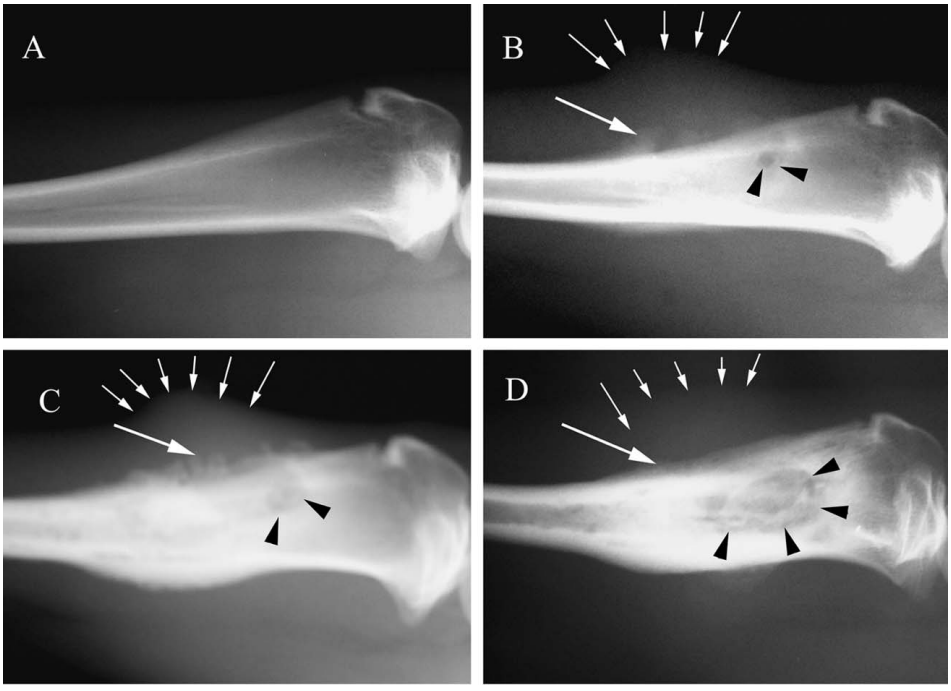

Fig 2A–D. (A) A serial radiograph of the goat tibia before inoculation is shown. (B) The periosteal response is established at 2 weeks and becomes more sclerotic and well defined by 12 weeks (large arrows). An extensive soft tissue response (small arrows), which gradually resolves, also is shown. (C) This radiograph taken 6 weeks after inoculation shows how the size of the metaphyseal radiolucency increases from 2 weeks to 12 weeks (black arrowheads). (D) A sequestrum is seen in the metaphyseal radiolucency 12 weeks after inoculation.

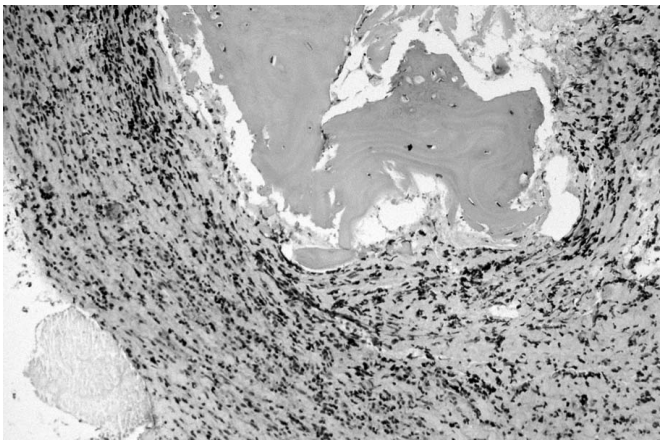

Fig 3. Histologic evaluation of a tibial specimen shows the remnants of viable lamellar bone with associated fibrosis and extensive chronic inflammation. These findings are consistent with an acute and chronic osteomyelitis (Stain, hematoxylin and eosin; original magnification, × 200).

rate of recovery of the infecting organism (82%). The goat model also has advantages based on cost, food consumption, and lack of aggressive behavior. These factors confirm our intention of developing the goat as a reasonable model of human osteomyelitis.

Beginning with Rodet in 1884, researchers have produced septic lesions resembling osteomyelitis in animals to varying degrees.[7–9] In some models, these lesions have been appropriate to chemotherapeutic interventions but did not lead to radiographic evidence of clinical osteomyelitis. As a result, these models have a limited role in the evaluation of surgical treatments. Rabbits and guinea pig models are limited because of their susceptibility to the toxic effects of antibiotic therapy. High-dose long-term therapy with some antibiotics has been associated with mortality and idiopathic diarrhea or pseudomembranous colitis.[4] Rats and chicks are reliable models of osteomyelitis and are inexpensive, but their small size has made the study of operative interventions prohibitive. Fitzgerald[2] developed a canine model of osteomyelitis to evaluate standard surgical procedures such as sequestrectomy and saucerization, but these animals are aggressive, and the use of this model is restricted by some funding organizations. Kaarsemaker et al[3] developed a sheep model for osteomyelitis. In their series, there was a high rate of complications. Before institution of immediate postoperative antibiotics, seven of the first 12 sheep died from fatal sepsis. Four other sheep, three of which had fatal sepsis, received antibiotics during the first day but not immediately after surgery. Even among the 36 sheep that did receive postoperative antibiotics, one died of sepsis, two had a slow-virus infection not related to the bacteria, and two others had pathologic fractures. Overall, 15 of 52 (29%) sheep had a major complication leading to their demise. Additionally, sheep are not as readily available in some regions, are more expensive to buy and to maintain, and can require more personnel because of their larger size. Three large animal distributors confirmed that goats were at least $50 less expensive than sheep (Animal Biotech Industries, Inc., Danboro, PA; Archer Farms, Inc., Darlington, MD; Nebeker Ranch, Los Angeles, CA).

Staphylococcus aureus was chosen for this study because of its prevalence in clinical osteomyelitis[1] and its use in varied investigations evaluating chemotherapeutic and surgical therapies.[5,11] The concentration of 4×10^9 CFU/mL was chosen after evaluation of a previous study in rats[6] and the unsuccessful attempt at obtaining osteomyelitis in a previous pilot study where the concentration of 4×10^8 CFU/mL was used.[3] The type of osteomyelitis developed in this goat model is classified as localized osteomyelitis according to the clinical staging system of Cierny et al,[1] which is a full thickness, cortical sequestration or cavitation or both. Localized osteomyelitis usually occurs after trauma and often has the combined features of medullary and superficial osteomyelitis resulting from an extension of either of the two entities.

Only one animal had a febrile episode (103.8°F) soon after inoculation that quickly subsided. One dose of cefazolin was given intravenously 1 hour after osteomyelitis inoculation to prevent fatal sepsis, which was noted in another animal study.[3] Neither leukocyte nor hemoglobin values changed from before inoculation compared with when the model was established. Monitoring these values may be of little value and unnecessary in future investigations with this particular model. All animals were fully weightbearing by postoperative Day 72 despite an initial period of limping.

Despite the success of this experiment in establishing osteomyelitis in the goat, there were several disadvantages associated with this animal model. First, histologic evaluation did not confirm osteomyelitis in one of the specimens. The lack of histologic characteristics of osteomyelitis in this specimen may have been related to sampling error because the histologic analysis did not reveal any bone fragments. Cultures from this specimen did grow the infecting S. aureus. Five other specimens did not have positive cultures but had histologic and radiographic osteomyelitis. This, again, might be related to sampling error or inadequate culturing techniques or both. Two of the 28 goats had complications at the end point of the study and died during the induction process. This was attributed to the sedating agent, xylazine. Neither of these deaths was related to the model or to the osteomyelitis. Subsequent to these cases, that agent was removed from our anesthetic protocol. The animals were followed up for a maximum of

4 months, there was no control or sham operation, and, because the disease was introduced in a hindlimb of a four-legged animal, the limb was not subject to the same weightbearing stresses as in a human.

We offer a consistent model for the study of chronic osteomyelitis that is also large enough for operative procedures, tolerant of a single generation cephalosporin, and has advantages over the sheep and canine model based on animal cost, feed costs, and daily care costs. This model is reproducible, adheres to general criteria for a satisfactory animal model for osteomyelitis, and seems useful for comparing alternative treatment approaches.

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