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Effectiveness of Local Antibiotic Delivery with an Osteoinductive and Osteoconductive Bone-Graft Substitute

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Background: The morbidity associated with open fractures and open fracture treatment is well established. An osteoinductive and osteoconductive bone-graft substitute that prevents infection would decrease the number of procedures required to treat contaminated fractures by eliminating the need for surgical removal of cement beads and perhaps autograft harvest. We hypothesized that the combination of tobramycin-impregnated calcium sulfate pellets and demineralized bone matrix would prevent the establishment of infection in a contaminated fracture model.

Methods: A unicortical 12-mm-diameter defect was created in the proximal tibial metaphysis of twenty-nine Spanish goats. After contaminating the wounds with an infective dose of Staphylococcus aureus, we divided the animals into four groups. The negative control group received no treatment, the positive control group received tobramycin-impregnated polymethylmethacrylate beads, the demineralized bone matrix group received 2.5 mL of demineralized bone matrix, and the experimental group received tobramycin-impregnated calcium sulfate pellets with 2.5 mL of demineralized bone matrix. Radiographs were made and intraosseous tissue cultures were performed on postoperative day 21.

Results: The cultures showed no evidence of intramedullary infection in the experimental or the positive control group, but they were positive for Staphylococcus aureus in six of the seven goats in the negative control group and seven of the eight goats in the demineralized bone matrix group.

Conclusions: The combination of tobramycin-impregnated calcium sulfate pellets and demineralized bone matrix was effective in preventing intramedullary Staphylococcus aureus infection in a contaminated goat fracture model.

Clinical Relevance: The local delivery of antibiotic with growth enhancers can prevent the establishment of intramedullary infection in association with open fractures. Such a combination therapy could potentially eliminate the need for surgical removal of cement beads and reduce the number of autografts harvested, thereby reducing the morbidity of open fracture treatment.
Therefore, we hypothesized that the combination of tobramycin-impregnated sulfate pellets and demineralized bone matrix could be used as a local antibiotic delivery system that is osteoinductive and osteoconductive as well as antimicrobial.

The purpose of this study was to evaluate the efficacy of a locally delivered antimicrobial with bone-graft substitute in the prophylactic treatment of infection in an open fracture. We hypothesized that the combination of tobramycin-impregnated calcium sulfate pellets and demineralized bone matrix would prevent the establishment of infection in a contaminated fracture model.

Materials and Methods

Animal Handling

The Institutional Animal Care and Use Committee approved all experiments and animal care procedures. All procedures were conducted in an animal facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care, and all were performed in accordance with the National Institutes of Health guidelines for care and use of laboratory animals.

Forty-eight Spanish goats with a weight range of 37 to 50 kg (mean and standard deviation, 42 ± 4 kg) were initially enrolled in the study; sample-size estimation with 81% power identified a requirement for twelve animals per group for the main experimental study.

All goats were housed in runs in a climate-controlled facility and were fed commercial food and water ad libitum. The goats were tested for tuberculosis, brucellosis, and Q fever, and they were observed for ten to fourteen days prior to the study to allow for environmental changes and to exclude the possibility of preexisting disease. A veterinarian examined each goat prior to commencement of the protocol.

Surgical Technique

The goats were not fed for forty-eight hours prior to the surgery, and water was withheld for twelve hours. After adequate regional and general anesthesia was achieved, the right lower extremity was prepared with chlorhexidine gluconate and draped in a sterile fashion. To avoid confounding variables, no preoperative intravenous antibiotics were given. A 2.5-cm longitudinal skin incision was made over the medial aspect of the proximal metaphyseal region of the tibia, centered at a point approximately 2 cm medial and 2 cm distal to the tibial tubercle. After the periosteum was elevated, a unicortical, 12-mm circular defect was produced with a coring reamer. A thrombin-soaked gelatin sponge was used to assist in medullary hemostasis. The osseous defect was inoculated with an aliquot of bacteria (30 µL of solution with a mean of 3.14 × 10^6 CFU/mL of Staphylococcus aureus). Thirty microliters of a 10^5 CFU/mL solution of bacteria has been shown to be sufficient to cause infection without producing sepsis in >70% of nontreated animals. The bacterial strain used was American Type Culture Collection (ATCC) 29213 (Manassas, Virginia), which was further modified by our institution to be resistant to streptomycin. Aliquots of Staphylococcus aureus were plated on streptomycin plates. The isolates with the highest concentration were recovered and were grown overnight in brain-heart infusion broth. This process was repeated twice more, and after the third pass the mutant was streaked on plates containing various concentrations of streptomycin (50 to 1000 µL/mL) to ensure resistance.

Before the study was started, the forty-eight goats were randomized into four groups of twelve animals each. A preplanned interim statistical analysis, which our institution's animal care committee had requested to conserve animals if statistical significance was achieved, showed that significance had been achieved midway through data collection. Thus the study was halted, resulting in the groups containing six, seven, or eight goats. The negative control group (seven goats) received no treatment. In the positive control group (six goats), fifteen to nineteen handmade tobramycin-impregnated polymethylmethacrylate beads (Palacos; Biomet, Warsaw, Indiana), resulting in a mean dose of 113.5 ± 7.3 mg of tobramycin sulfate, were used to fill the bone defect of each goat. The inconsistent dose and number of beads in this group was due to the slight variation in the sizes of the handmade beads as well as the need to adjust the numbers of beads to fill the metaphyseal voids of differently sized goats. The demineralized bone group (eight goats) had 2.5 mL of demineralized bone matrix (Allomatrix injectable putty; Wright Medical, Arlington, Tennessee) placed into the metaphyseal defect. The experimental group (eight goats) received fifteen pellets of 10% tobramycin-impregnated calcium sulfate (OSTEOSET T; Wright Medical) with 2.5 mL of demineralized bone matrix. The fifteen pellets of OSTEOSET T had a total of 160 mg of tobramycin sulfate.

Wound Evaluation

The animals were followed daily for twenty-one days for clinical signs of infection. Abscess formation was followed clinically by measuring leg circumference. Abscesses that increased in size over three days were relieved with aspiration or incision and drainage. Discharge from abscesses was sent for culture and bacterial identification. At the first sign of wound drainage, the discharge was also cultured, and bacterial species were identified. All Staphylococcus aureus isolates were tested for streptomycin resistance to determine if the cultured strain was identical to the original bacterial inoculate. If any animal demonstrated discomfort or distress during the three-week observation period, a 100-μg/hr fentanyl citrate patch was placed on the skin of the neck area and secured with elastic bandaging tape under the supervision of a veterinarian. On postoperative day 21, all of the goats were killed and necropsy studies were performed as described below.

Wound Grading System

Clinical signs of wound infection included erythema, inflammation, and purulent drainage. After the dressing was removed on postoperative day 4, three independent examiners graded each wound daily. The graders were blinded to the treatment groups for the duration of the study. The clinical grading system that was used had been established in a previ-
The wound was assigned a score of 0 when there were no signs of contamination or swelling; a score of 1 when there was inflammation, swelling, or serous drainage without frank purulence; and a score of 2 when there was frank purulence at the wound site or purulent discharge on aspiration or incision and drainage. A total score for each wound was calculated by adding the scores assigned by each of the three observers each day for twenty-one days. The clinical determination of infection was defined by a score of at least 4 on two consecutive days. Hence, a wound had to exhibit purulent drainage, as identified by two of the three examiners, for two consecutive days to be considered infected.

**Necropsy and Microbiologic Analysis**

On postoperative day 21, the goats were killed, the treated hindlimb was disarticulated at the hip, and anteroposterior and lateral radiographs were made. Soft tissue was removed from the tibia, and the osseous defect was transected at its midportion with a Gigli saw. Culture swabs were obtained by swabbing the canal proximal and distal to the defect. A number-5 surgical curet (to obtain 0.5 g of tissue) was used to harvest marrow and trabecular tissue from the canal. Finally, a 2-cm portion of the tibia encompassing the osseous defect was removed with a sagittal saw.

The tissue and swab samples were sent for culture and identification of the bacterial species. Each *Staphylococcus aureus* isolate was tested for streptomycin resistance to determine whether it was the same strain as the initial inoculum.

**Outcome Measure**

The outcome measure used to identify a deep wound infection was the recovery of the streptomycin-resistant *Staphylococcus aureus* strain ATCC 29213 from intramedullary cultures at twenty-one days. The threshold for infection was set at 10^6 CFU/g of marrow. Cultures in which bacteria were present but the count was <10^5 CFU/g of marrow were considered to be contaminated. If the quantitative analysis identified a bacteria count of between 10^5 and 10^6 CFU/g of marrow in the final tissue culture, the clinical score was used to determine whether an infection was present (i.e., the wound had to be considered infected by our clinical scoring criteria to be considered infected).

**Statistical Analysis**

Analysis of variance was used to ensure that the groups were comparable in terms of body weight and amount of inoculation (CFU/g of marrow). A nonparametric median test was used to test for differences between treatment groups. Clinical scores were compared between groups with use of a chi-square test. When global differences were detected, a Bonferroni adjustment was used for error correction in order to determine the significance of subsequent comparisons.

### Results

None of the animals displayed signs of systemic sepsis (decreased activity or alertness, lack of appetite, or fever). There was no significant difference between groups with respect to the mean body weight of the goats (p = 0.96) or the mean amount of bacterial inoculum (p = 0.86).

In the negative control group, the wound scores ranged from 0 to 6 during the clinical evaluation period. Purulent drainage developed in one goat, which received a wound score of 6. Four goats received a wound score of 3; one goat, with negative cultures, received a score of 0 (normal); and another goat, with positive cultures, received a score of 0. (see Appendix). All of the goats in this group showed evidence of periosteal reaction on the final radiographs. Gross pathologic examination demonstrated necrosis and abscess formation in five of the seven goats. Cultures at twenty-one days confirmed intramedullary infection with streptomycin-resistant *Staphylococcus aureus* in six of the seven goats. The mean count in the group was 2.2 x 10^6 ± 3.3 x 10^5 CFU/g (Table I).

In the positive control group, all six goats had wound healing without any signs of infection during the clinical evaluation period, and all received a wound score of 0. None of the final radiographs showed evidence of periosteal reaction, and gross pathologic examination showed no signs of infection. Cultures did not demonstrate bacteria in the intramedullary tissues of any of the goats in this group.

In the demineralized bone matrix group, purulent discharge developed, and the wound score was 6, in six of the eight goats. All eight goats showed evidence of periosteal reaction on radiographic examination. Gross pathologic analysis demonstrated abscesses, necrosis, and draining sinuses in seven of the eight goats. Cultures at twenty-one days confirmed intramedullary infection with streptomycin-resistant *Staphylococcus aureus* in seven of the eight goats. The mean count was 1.3 x 10^6 ± 2.3 x 10^5 CFU/g (Table I). One goat had purulent discharge that was positive for streptomycin-resistant *Staphylococcus aureus* on culture during the clinical evaluation period, but the final culture demonstrated *Strepto-*
**Coccus viridans** in the intramedullary tissue.

In the experimental group, four of the eight goats had early, culture-negative, serous discharge for two to six days during the clinical evaluation period. Two goats in this group had superficial wound infections; one was infected with non-hemolytic Streptococci, and one infection resolved after removal of a small superficial eschar. Radiographic examination revealed periosteal elevation in six of the eight goats; however, the degree of reaction was qualitatively less than the reaction seen in both the negative control and the demineralized bone matrix group. Gross pathologic examination demonstrated incorporation of the demineralized bone matrix and calcium sulfate pellets without evidence of infection (Fig. 1). There was no growth of any bacteria on culture of the intramedullary tissues from the eight goats in this group.

All goats in both groups that had received tobramycin had negative cultures for intramedullary bacteria at twenty-one days (Table I). There were significant differences between the treatment groups in terms of the number of CFU/g (p < 0.0001). The positive control group had a significantly lower number of CFU/g than the negative control group (p = 0.018) and the demineralized bone matrix group (p = 0.011). Similarly, the experimental group showed a significantly lower number of CFU/g than either the negative control (p = 0.0066) or the demineralized bone matrix group (p = 0.0006). With the numbers available, the negative control and demineralized bone matrix groups were not significantly different from one another in this regard (p = 0.46).

The demineralized bone matrix group had a significantly higher number of clinical infections (a score of at least 5 on two consecutive days) than did the experimental group (p = 0.006), the positive control group (p = 0.01), or the negative control group (p = 0.02). There was no significant difference in the wound score between the experimental group and the positive control group (p = 0.09).

**Discussion**

The current study demonstrated that an antibiotic-impregnated bone-graft substitute can be used effectively to treat infection in a contaminated fracture model. Previous animal studies and human clinical trials have shown tobramycin-impregnated calcium sulfate pellets to be effective in the treatment of osteomyelitis. Since biodegradable antibiotic drug delivery systems are effective against osteomyelitis, it stands to reason that they might also be effective as prophylaxis. Previous work at our institution showed that tobramycin-impregnated calcium sulfate pellets by themselves provided effective prophylaxis in a similar goat model. However, calcium sulfate is only osteoconductive, and, in a canine model, it took twenty-four weeks for it to stimulate as much bone growth as was produced in defects treated with autogenous bone graft. The combination of calcium sulfate pellets and demineralized bone matrix is osteoconductive and osteoinductive, and it stimulated as much bone growth as did autograft in just six weeks in the same

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**Fig. 1**

Necropsy specimen consisting of the proximal part of the tibia from a goat in the experimental group. The tibia is cut through the proximal metaphysis at the cortical defect. Note the healthy-appearing marrow and the incorporation of calcium sulfate pellets into the marrow.
model. We hypothesized that, with the addition of tobramycin, the combination should be antimicrobial as well, but this combination had never been tested in a contaminated fracture model, to our knowledge.

In the contaminated fracture model used in this study, the combination of tobramycin-impregnated calcium sulfate pellets and demineralized bone matrix did not differ significantly from tobramycin-impregnated cement beads with regard to its ability to prevent the establishment of *Staphylococcus aureus* infection. Therefore, this combination could decrease the morbidity of open fractures by eliminating the need for removal of surgical beads and by reducing the number of autografts harvested.

One limitation of this study was that the degree of bone-healing was not compared between groups. This model was developed to determine the ability of a treatment to prevent an infection in a contaminated bone defect, which is a necessary first step in assessing the usefulness of a treatment for an open fracture. However, the ability of this combination to promote healing of open fractures is, as yet, untested. Another limitation of this study is that the experimental group received more tobramycin sulfate than did the positive control group because the calcium sulfate pellets had a higher concentration of tobramycin sulfate than did the handmade beads (10% compared with 4% by weight). Handmade beads are the current standard of care, and the current recommendation is to mix 2.4 g of tobramycin with 40 g of polymethylmethacrylate, which produces the above concentration. This was not intended to be a definitive dose-response study; still, the higher concentration and load of tobramycin that the experimental group received may have biased that group's success. However, this concentration does not adversely increase serum tobramycin levels.

An important observation was the serous drainage that occurred for two to six days in the experimental group. Similar drainage has been observed in clinical trials, in which the investigators noted that the drainage halted when there was larvae drainage has been observed in clinical trials, in which the investigators noted that the drainage halted when there was significant fibrin. This drainage may explain the two superficial wound infections in the experimental group. Additional study will focus on the effect of this combination on bone-healing and compare this approach with other current standards of care such as the use of antibiotic cement beads in combination with intravenous antibiotics.

### Appendix

A table presenting the wound scores and culture results for all study animals is available with the electronic versions of this article, on our web site at jbjs.org (go to the article citation and click on “Supplementary Material”) and on our quarterly CD-ROM (call our subscription department, at 781-449-9780, to order the CD-ROM).  

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