### Title and Subtitle

**Development of Chronic Mandibular Osteomyelitis in a Miniswine Model**

### Authors

A.L. Patterson, R.H. Galloway, J.C. Baumgartner, T.S. Barsoum

### Performing Organization Name(s) and Address(es)

Dental Research Detachment,
Walter Reed Army Inst. of Research
6825 16th St. N.W., Wash. D.C. 20307-5300

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### Abstract

Previous attempts to develop a reproducible model of chronic mandibular osteomyelitis have met with little success. In this study, osteomyelitis was produced in the mandibles of eight adult yucatan miniswine by the intramedullary application of sodium morrhuate, *Staphylococcus aureus*, and either polymethylmethacrylate bone cement or bone wax. At 8 weeks postinfection, the mandible was surgically debried and specimens were obtained for culture. Although all of the animals developed clinical evidence of osteomyelitis that was supported by positive cultures, the original organism (*S. aureus*) was recovered only from those animals whose bone wax had been used to seal the cortical defects. This animal model may be useful for evaluating newer treatment modalities of chronic osteomyelitis.

### Subject Terms

Mandibular osteomyelitis - Miniswine model - *Staphylococcus aureus*.
Development of Chronic Mandibular Osteomyelitis in a Miniswine Model

A.L. PATTERSON, DDS,* R.H. GALLOWAY, DDS,† J.C. BAUMGARTNER, DDS, PHD,‡ AND I.S. BARSOUM, PHD§

Previous attempts to develop a reproducible model of chronic mandibular osteomyelitis have met with limited success. In this study, osteomyelitis was produced in the mandibles of eight adult Yucatan miniswine by the intramedullary application of sodium morrhuate, Staphylococcus aureus, and either polymethylmethacrylate bone cement or bone wax. At 8 weeks postinfection, the mandibles were surgically debrided and specimens were obtained for culture. Although all of the animals developed clinical evidence of osteomyelitis that was supported by positive cultures, the original organism (S aureus) was recovered only from those animals where bone wax had been used to seal the cortical defects. This animal model may be useful for evaluating newer treatment modalities for chronic osteomyelitis.

Previous studies dealing with the development of a reproducible model of chronic mandibular osteomyelitis in rabbits and rhesus monkeys have met with limited success. Most successful reports of experimental osteomyelitis have involved infections of long bones, primarily in rabbits and dogs. The mandible, however, appears to be more resistant to infection than long bones, mainly because its rich vascular supply reduces the chances of establishing an infection in this location. Nevertheless, there is a need to develop such a model to gain a better understanding of the disease process and to evaluate newer methods of treatment.

Miniatuerc swine are well suited for dental research because they are omnivorous, possess both deciduous and permanent teeth, display both chewing and biting actions of the mandible, and possess an immune system similar to humans. Fitzgerald described a surgical technique of inducing chronic osteomyelitis in the tibia of dogs using a combination of Staphylococcus aureus and polymethylmethacrylate (PMMA) bone cement. In the present study a chronic mandibular osteomyelitis model was developed in miniature swine by the intramedullary application of a mild sclerosing agent (sodium morrhuate), S aureus as the infecting agent, and either PMMA bone cement or bone wax as a foreign body.

Materials and Methods

Animals

Eight mature, female Yucatan miniswine, 2 to 5 years of age (Charles River, Willington, MA; and Buckshire Corp, Perkasie, PA), each weighing between 68 and 95 kg, were used in these experiments to ensure that the permanent dentition was fully erupted and that the bony architecture of the mandible was mature. The animals were housed in individual cages and provided a standard laboratory diet consisting of Purina Minipig Chow no. 5081 (Purina, St Louis, MO). The experiments were conducted according to the principles set forth in the “Guide for Care and Use of Laboratory Animals.”

* Colonel, Dental Corps, Department of Oral and Maxillofacial Surgery, Walter Reed Army Medical Center, Washington, DC.
† Lieutenant Colonel, Dental Corps, Chief Surgery Branch, US Army Institute of Dental Research, Walter Reed Army Medical Center, Washington, DC.
‡ Associate Professor, Chairman, Department of Endodontology, School of Dentistry, Oregon Health Sciences University, Portland, OR.
§ Microbiologist, Microbiology Branch, US Army Institute of Dental Research, Walter Reed Army Medical Center, Washington, DC.

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Address correspondence and reprint requests to Dr Barsoum: US Army Institute of Dental Research, Walter Reed Army Medical Center, Washington, DC 20307-5300.

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Bacterial Inoculum

*S. aureus*, ATCC strains 6538P, 25923, and 29213 (American Type Culture Collection, Rockville, MD), were grown in brain heart infusion (BHI; DIFCO Laboratories, Detroit, MI) broth at 37°C overnight. Colony-forming units (CFU) were determined by performing serial dilutions of the bacterial cultures and plating each dilution on sheep blood agar plates. Bacteria in the logarithmic phase of growth were aliquoted in BHI containing .25% (w/v) agarose (DIFCO) and kept frozen at -70°C until used. On the day of surgery, individual frozen vials of each bacterial suspension were thawed and kept on ice until used.

Surgical Procedures

The animals were premedicated with atropine (.5 mg/kg; subcutaneously) and then sedated with intramuscular administration of ketamine (.45 mg/kg), and xylazine (.45 mg/kg). After adequate sedation was achieved, intravenous access was obtained via the ear vein and an induction dose of Biotal (5 to 8 mg/kg; Bio-Ceutic, St Joseph, MO) was administered. Oral endotracheal intubation was then accomplished and anesthesia maintained with \( N_2O/O_2/\text{Forane} \) (Ana-Quest Inc, Liberty Corner, NY). The lateral mandible and submandibular areas were prepared and draped in a sterile fashion and an 8 to 10-cm incision was made in the posterior submandibular region. The incision was sharply deepened in layers through the skin and subcutaneous tissue to the level of the pterygomasseteric muscle sling, which was then sharply transected and reflected superiorly to expose the posterior and lateral body of the mandible.

An 8-mm trephine was used to penetrate the lateral cortical plate approximately 1 cm above the inferior border (Figs 1, 2). The bony plug was removed and a bent, flat-blade electrocautery tip was inserted into the medullary cavity to control bleeding and to produce some areas of necrotic cortical and cancellous bone. A gauze sponge saturated with 5% sodium morrhuate (Scleromate, Palisades Pharmaceuticals, Tenafly, NJ) was packed into the cavity and allowed to remain in situ for 15 minutes. The gauze sponge was removed and the bone cavity was copiously irrigated with saline (Fig 3). One milliliter of the bacterial suspension (10^8-10^9 CFU *S. aureus*) was then injected into the medullary canal and the cavity and trephine hole were filled with either PMMA bone cement or bone wax. The soft tissue was then irrigated and closed in layers with 2-0 Dexon absorbable sutures (Davis and Geck, Inc, Manati, PR). The animals were returned to their cages and were periodically monitored over the next 8 weeks for clinical evidence of osteomyelitis and other potential postoperative complications. At 8 weeks postinfection, the animals were anesthetized as before and the surgical site was reentered through the previous incision. Surgical debridement was performed and specimens were collected aseptically for culture as described below. Four weeks following surgical debridement (12 weeks postinfection), specimens were again obtained from all miniswine for bacterial culture.

Identification of Bacterial Isolates

Specimens obtained at 8 and 12 weeks postinfection were processed for both aerobic and anaerobic bacterial isolation using standard microbiologic methods. Purtulent material (when present) was aspirated into a 10-mL syringe and transported immediately to the bacteriology laboratory. Bone and tissue specimens were divided into portions and placed in sterile empty tubes.
and in tubes containing thioglycolate medium. Soft tissue and bone specimens were homogenized by grinding in a sterile mortar and pestle. Serial tenfold dilutions were made in sterile saline and 1 mL of each dilution was plated in duplicate on sheep blood agar and mannitol salt agar plates. Specimens were also inoculated on Brucella agar and Columbia blood agar plates and incubated in an anaerobic chamber (Model 1025, Forma Scientific, Marietta, OH) for 72 to 96 hours. Isolated colonies from culture specimens were identified on the basis of colony morphology, blood hemolysis, growth on selective media, and Gram stain. Identification was confirmed using biochemical tests for the appropriate organisms (API System, Analytab Products, Plainview, NY).

Results

All miniswine developed clinical evidence of mandibular osteomyelitis by 8 weeks postinfection. This was characterized by suppuration, swelling, persistent induration, and proliferative periostitis. There did not appear to be any significant difference with respect to the severity of the osseous infection between the two animals where PMMA was used to seal the trephine hole and the six miniswine whose defects were filled with bone wax. Copious amounts of purulent material were frequently found at the time of surgical debridement and, in several cases, exfoliated, necrotic bone was found (Fig 4). Bone wax was encountered in soft tissue abscesses located adjacent to the mandible. In other cases, however, the infectious process was limited to the medullary cavity. In those instances where the bone wax had been forced into the soft tissue, there was exuberant growth of reactive bone both lateral to the mandible and within the original trephine hole. It was, therefore, necessary to remove large amounts of reactive bone to gain access to the medullary cavity for obtaining specimens for culture.

*S. aureus* was recovered at 8 weeks postinfection from all six miniswine where bone wax had been used to seal the trephine hole but not from the two PMMA animals (Table 1). Surgical debridement did not significantly alter the course of the infectious process. As shown in Table 1, at 4 weeks following surgical debridement (12 weeks postinfection), *S. aureus* was again recovered from five of six miniswine in the bone wax group. All three strains of *S. aureus* used in this study were able to produce mandibular osteomyelitis in the miniswine.

The major determining factor in the ability to recover the original infecting organism (*S. aureus*) in this experimental model of mandibular osteomyelitis seemed to be the presence of bone wax, rather than PMMA bone cement, in the trephine hole. Although *S. aureus* was not recovered uniformly from all animals, nevertheless, specimens obtained for culture at both 8 and 12 weeks postinfection were positive for bacterial growth. As can be seen in Table 2, a variety of both aerobic and anaerobic microorganisms also were recovered from soft tissue and/or bone specimens, several of which were of veterinary origin.

Discussion

Several studies have been reported in the literature dealing with the development of an animal model of mandibular osteomyelitis. Kondell et al. injected a mild sclerosing agent (5% sodium morrhuate) and *S. aureus* into the mandibles of 20 rabbits and allowed the infection to become established over 2 weeks. The success rate of inducing a chronic mandibular osteomyelitis with this model, however, was only 60%. Aside from
the poor degree of reproducibility, this model was also associated with a high mortality rate.

Triplett et al. created sagittal fractures in the mandibles of rabbits and cauterized the vessels in the mandibular canal to induce vascular compromise. The fracture sites were then inoculated with Bacteroides melaninogenicus and the cortical plates were loosely approximated with wires. Although all of these animals developed clinical evidence of chronic osteomyelitis by 8 weeks, cultures of these lesions showed a mixed flora in all cases. The infecting organism (B melaninogenicus) could not be recovered from the vast majority of these lesions. The investigators attributed this to the fact that the fractures communicated with the oral cavity, which resulted in overgrowth by the organisms from the normal oral flora.

A more recent study in the literature involving the development of an animal model of chronic osteomyelitis was reported by Wannfors and Hammarstrom. In this study, an infection was induced in the lateral incisor of five monkeys by implanting dental plaque into the pulp chambers. Three weeks later, these teeth were extracted and the infected tooth roots were surgically implanted into the mandibles through the alveolar socket of a freshly extracted mandibular premolar. After 6 months, the monkeys were killed and the mandibles were removed for histologic evaluation. Although at 8 weeks postinfection all of the animals developed clinical evidence of osteomyelitis, which was supported by positive cultures of debrided material from the lesions, the original infecting organism (S aureus) was only recovered from the six animals where bone wax was used to seal the trephine hole. These findings are in agreement with Nelson et al. who reported on the promotional effects of bone wax as a foreign body in experimental S aureus osteomyelitis in the femur of rats. The inability to recover S aureus from the two animals in the PMMA group may be due to the possibility that the organisms did not survive the high temperatures generated during the polymerization of the PMMA bone cement.

To our knowledge, this study is the first to report on the use of miniature swine as an experimental animal model of chronic mandibular osteomyelitis. As noted earlier, previous investigators have found miniswine to be especially well suited for dental research because their dentition, as well as their immune system, is similar to those of humans. Although the cost of purchasing and housing miniature swine is significantly higher than many other laboratory animals, nevertheless, the large size of the miniswine mandible is advantageous for evaluating newer experimental treatment modalities for chronic mandibular osteomyelitis, especially those requiring surgical intervention.

Table 1. Mandibular Osteomyelitis in Miniswine at 8 and 12 Weeks Postinfection

<table>
<thead>
<tr>
<th>Animal No</th>
<th>S aureus ATCC Strain</th>
<th>Foreign Body</th>
<th>Osteomyelitis* 8 wk</th>
<th>Osteomyelitis* 12 wk</th>
<th>S aureus 8 wk</th>
<th>S aureus 12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-107</td>
<td>6538P</td>
<td>PMMA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS-268</td>
<td>6538P</td>
<td>PMMA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>MS-661</td>
<td>25923</td>
<td>Bone wax</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>MS-70-5</td>
<td>29213</td>
<td>Bone wax</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>MS-14-5</td>
<td>6538P</td>
<td>Bone wax</td>
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<td>+</td>
</tr>
<tr>
<td>MS-9-3</td>
<td>6538P</td>
<td>Bone wax</td>
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<td>+</td>
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<td>MS-78</td>
<td>6538P</td>
<td>Bone wax</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>MS-79</td>
<td>6538P</td>
<td>Bone wax</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviation: ATCC, American Type Culture Collection (Rockville, MD).
* Clinical evidence of bone infection based on presence of suppuration, swelling, persistent induration, and/or proliferative periostitis.

Table 2. Aerobic and Anaerobic Bacteria Other Than S aureus Isolated From Miniswine With Mandibular Osteomyelitis

<table>
<thead>
<tr>
<th>Aerobic Organisms</th>
<th>Anaerobic Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Bacteroides melaninogenicus</td>
</tr>
<tr>
<td>Staphylococcus hominis</td>
<td>Clostridium sporogenes</td>
</tr>
<tr>
<td>Staphylococcus sciuri</td>
<td>Peptostreptococcus anaerobius</td>
</tr>
<tr>
<td>Streptococcus acidominimus</td>
<td></td>
</tr>
<tr>
<td>Streptococcus cremoris</td>
<td></td>
</tr>
<tr>
<td>Streptococcus thermophilus</td>
<td></td>
</tr>
</tbody>
</table>

intramedullary application of a mild sclerosing agent, S aureus, and a foreign body (PMMA or bone wax).


