# Local treatment of chronic osteomyelitis with surgical debridement and tigecycline-impregnated calcium hydroxyapatite: an experimental study

Mahir Kaya, PhD,[a] Göksel Şimşek-Kaya, DDS, PhD,[b] Nesrin Gürsan, MD,[c] Ekrem Kireççi, PhD,[d] Ertunç Dayı, DDS, PhD,[e] and Betül Gündoğdu, MD,[f] Erzurum and Kahramanmaraş, Turkey
ATATURK UNIVERSITY, KAHRAMANMARAS SUTCUIMAM UNİVERSITY

**Objective.** The aim of this study was to determine the efficacy of tigecycline-impregnated hydroxyapatite in the local treatment of chronic osteomyelitis experimentally induced in rat tibias with methicillin-resistant *Staphylococcus aureus*.
**Study Design.** Monocortical defects were established in the left tibias of 32 adult Wistar albino rats. Five rats were randomly selected and injected intramedullarly with saline solution (group 1), whereas chronic osteomyelitis was induced in other rats by intramedullary injection of *S. aureus*. Infected rats were then randomized and divided into 4 groups: group 2, no further treatment; group 3, debridement only; group 4, debridement followed by implantation of calcium hydroxyapatite; and group 5, debridement followed by implantation of tigecycline-impregnated calcium hydroxyapatite. On day 21 after induction, all rats in groups 2-5 showed signs of osteomyelitis. Rats in groups 1 and 2 were killed on day 21 after induction, whereas rats in groups 3, 4, and 5 underwent debridement surgery on day 21 after induction and were killed 21 days after debridement surgery. Tibias were analyzed histopathologically and cultured for *S. aureus*.
**Results.** Compared with group 2, histopathologic disease severity scores in groups 3, 4, and 5 were 37%, 44%, and 83% lower, respectively. Nontreated infected rats had the highest bacteria count (mean $5 \times 10^5$ colony-forming units/g bone), and bacterial count was 26%, 29%, and 79% lower in groups 3, 4, and 5, respectively, compared with group 2.
**Conclusions.** Tigecycline-impregnated hydroxyapatite can have a potential in the treatment of chronic osteomyelitis of methicillin-resistant *S. aureus* origin, which may be considered as a therapeutic alternative by surgeons dealing with osteomyelitis. (Oral Surg Oral Med Oral Pathol Oral Radiol 2012;113:340-347)

Osteomyelitis is a progressive infection of the bone marrow and cortex that is usually staphylococcal in origin and that can be caused by trauma, nosocomial infection, and orthopedic[1] and maxillofacial surgery.[2] The pathogen *Staphylococcus aureus* is particularly threatening owing to its acquired resistance to methicillin and its capacity to lead to severe bone infections.[3] Although glycopeptides have long been used successfully to treat severe methicillin-resistant *S. aureus* (MRSA) infections, rising levels of heteroresistance and glycopeptide tolerance[4] have encouraged the search for newer agents.

Treatment of chronic osteomyelitis is expensive, lengthy, and difficult. Standard therapy generally covers debridement, dead space management, soft tissue reconstruction, and culture-directed antibiotics.[5] Antibiotics used to treat orthopedic infections are delivered either systemically through intravenous infusion or locally through the use of antibiotic-impregnated implant material. Disadvantages of intravenous antibiotic therapy include catheter failure, infection, systemic drug toxicity, and cost. In addition, bone necrosis associated with osteomyelitis may reduce vascularity, considerably lowering the effectiveness of systemic antibiotic therapy.[6,7] Antibiotic-impregnated implant materials are used to manage the dead space created by debridement surgery in patients with osteomyelitis and offer a number of advantages to intravenous antibiotherapy.[8] Antibiotic-impregnated polymethylmethacrylate (PMMA) beads and spacers are frequently used to locally treat debridement defects by supplying sustained[9] elevated concentrations of antimicrobial agents directly to the infected area without exerting the systemic effects or other limitations of intravenous therapy.[10] However, PMMA beads have the major disadvantage of requiring surgical removal once their antibiotics have been released.[11] Moreover, PMMA rapidly becomes coated

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[a]Assistant Professor, Department of Surgery, Faculty of Veterinary Medicine, Atatürk University.
[b]Assistant Professor, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Atatürk University.
[c]Professor, Department of Pathology, Faculty of Medicine, Atatürk University.
[d]Assistant Professor, School of Health, Kahramanmaraş Sütçüimam University.
[e]Professor, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Atatürk University.
[f]Assistant Professor, Department of Pathology, Faculty of Medicine, Atatürk University.
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with host proteins, such as fibrin, producing an environment ideal for bacterial colonization and infection from transient bacteremia.[12] Finally, implantation of a medical vehicle may compromise local immune response, because the host immune system directs its activity against the foreign implant instead of invading pathogens.

Considerable attention has been paid to biodegradable materials as alternatives to PMMA in an effort to reduce the likelihood of implant-associated infections and immune system reaction.[9] Various biodegradable materials have been studied with promising results, including high-molecular-weight biodegradable poly(D,L-lactide) cylinders, calcium hydroxyapatite (CHA), and polyglycolide beads.[13,14] CHA is a major component of compact bone, and the biocompatibility and close resemblance of CHA implants to natural hard tissue represent distinct advantages.[15] In addition, soft-tissue and bone defects are slowly filled with new tissue as the implant is gradually replaced by newly formed bone, thus eliminating the need for reconstruction.[8]

Tigecycline (formerly GAR-936) is a new glycylcycline that was licensed by the U.S. Food and Drug Administration in June 2005 for the treatment of complicated skin and intraabdominal infections. Tigecycline exhibits expanded broad-spectrum antibiotic activity. A number of comprehensive studies suggest that tigecycline is highly active in vitro against most common gram-positive and gram-negative pathogens, anaerobes, and atypical pathogens, including those that commonly exhibit resistance to multiple classes of antimicrobials. MRSA is one gram-positive bacteria susceptible to tigecycline.[16] By binding to the 30S ribosomal subunit, tigecycline obstructs entry into the aminoacyl transfer RNA acceptor site, thereby inhibiting protein synthesis and bacterial growth.[17] The aim of the present study was to investigate the efficacy of tigecycline-impregnated CHA in the local treatment of chronic osteomyelitis of MRSA origin induced experimentally in rats.

## MATERIAL AND METHODS

### Animals and management

A total of 32 male Wistar albino rats ~4 months in age and weighing an average of 245 g (190-290 g) were used in accordance with a protocol approved by local Ethical Committee on Experimental Animal Use and Care and in full compliance with the Helsinki Declaration of Animal Rights. Rats were placed in pairs in cages in a room with a 12-hour daylight/darkness cycle and an ambient temperature (23°C-28°C). Rats were fed chow, and water was provided ad libitum.

### Bacteria

A clinical isolate of MRSA that is sensitive to vancomycin and resistant to methicillin was used. Bacteria were incubated in a *Staphylococcus* medium (Oxoid, CM145) broth at 37°C for 24 hours. Colonies were taken directly from plates and diluted with 0.9% NaCl to obtain a McFarland 0.5 standard. After checking the density of the bacterial suspension by using a spectrophotometer (Ultraspec 2000; Pharmacia, U.K.), $1 \times 10^5$ colony-forming units (CFU)/100 μL inoculates were prepared for intramedullary administration.[18]

### Calcium hydroxyapatite

Calcium hydroxyapatite cement was obtained from BoneSource (Stryker Biotech Europe, Switzerland). Tigecycline-impregnated CHA was obtained by adding 2.0 g tigecycline (Tygacil 50 mg; Wyeth, USA) to 40 g CHA powder before implantation.

### Induction surgery and postinduction care

Chronic osteomyelitis was induced in 27 of 32 rats, according to the method described by Zak et al.[19] Under general anesthesia with xylazine (5 mg/kg) and ketamine (75 mg/kg), the left hind leg of each rat was shaved, the skin disinfected with povidone iodine, and a 1-cm longitudinal incision was made to the anteromedial aspect of the tibia. After subcutaneous and periosteal dissection, a 1-mm-diameter twist drill was used to create an intramedullary cavity in the patellotibial tendon to a depth of ~1 cm below the medullary canal, with the entry point between the tibial plateau and the tibial tuberosity. Through this aperture, 10 mL 5% sodium morphate (Scleromate; Glenwood, USA) was injected into the canal. Local irritation was induced by using morrhuate, a complex of fatty acids rich in arachidonic acid,[20] and 100 μL $1 \times 10^5$ CFU *S. aureus* was injected, followed by 0.2 mL 0.9% NaCl solution. The contaminated solution was allowed to spread throughout the medullary cavity, after surgically closing the fascia and skin layers.

On the remaining 5 rats (group 1, sham), similar procedures were followed except for inducing infection; instead, 0.1 mL saline solution was injected into the tibial defect. All animals were monitored for 3 weeks after the surgery to allow for the development of osteomyelitis without restricting their functional activity and administering systemic antibiotic to not confound the experimental outcome. Owing to invasive processes, rats were given analgesic (buprenorphine 0.05 mg/kg subcutaneously every 12 hours for 3 days).

### Treatment groups

Three weeks after induction surgery, infected rats were randomly distributed into 4 treatment groups: group 2

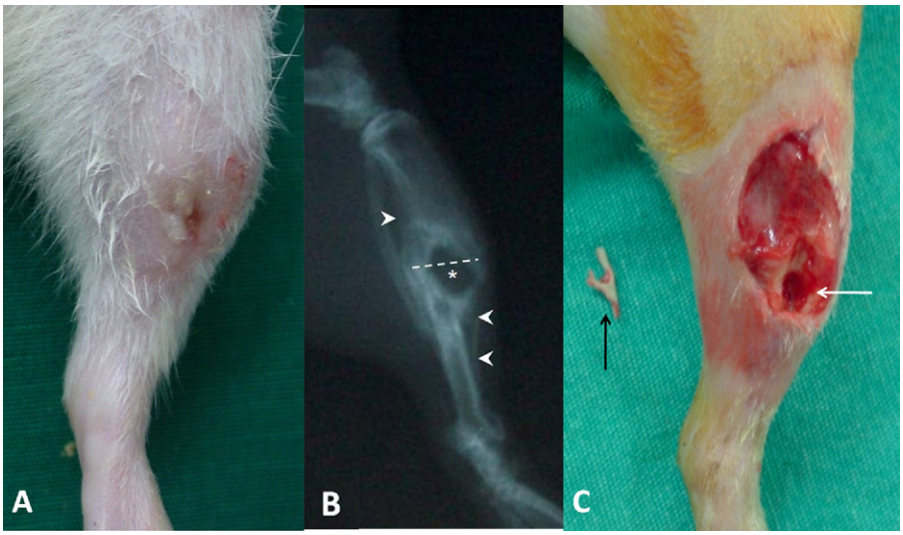



Fig. 1. Macropathologic, radiographic, and perioperative images of a rat in the debridement group. **A,** Macroscopic image shows sinus formation and pus discharge. **B,** Radiography shows grade 4 osteomyelitis, bone lucency (*asterisk*), diaphyseal deformation and widening (*dashed line*), and periosteal reaction (*arrowheads*). **C,** Another image from the same rat shows dead space (*white arrow*) and sequestrum (*black arrow*) after debridement surgery.

(n = 6), negative control, no treatment; group 3 (n = 7), debridement surgery only; group 4 (n = 7), debridement surgery and implantation of CHA without antibiotic impregnation; and group 5 (n = 7), debridement surgery and implantation of tigecycline-impregnated CHA.

### Debridement surgery and implantation

Debridement surgery was performed 21 days after induction surgery in groups 3-5. Using the surgical approach described above, the area of the intramedullary defect was exposed and swab cultures taken to confirm the successful induction of staphylococcal bone infection. Infected and necrotic soft tissue was removed to the greatest extent possible, and the canal was flushed with 100 mL 0.9% saline solution to remove debris and remaining bone marrow (Fig. 1, *C*). Plain CHA and tigecycline-impregnated CHA was packed into the dead space remaining after debridement in groups 4 and 5, respectively, and the fascia and skin layers were closed. Depending on the size of the defect, between 0.5 and 2 g CHA cement was implanted after debridement.

### Macroscopic examination

Following induction and debridement surgery, all rats were examined for inflammation and clinical signs, including redness, swelling, sinus formation, pus discharge, tibial deformation, lameness, and use of the left hind leg in walking.

### Radiographic evaluation

Radiograms (Diagnox Floatex Model; Meditronics, India) of the left tibias of all rats in groups 2-5 were taken 21 days after the induction surgery. Development of tibial osteomyelitis was confirmed based on the following parameters: periosteal reaction, diaphyseal widening, osteolysis, bone deformation, and sequestrum formation.

On day 21 after debridement surgery, radiograms were taken of rats in groups 3-5 and compared with pretreatment radiograms to evaluate the extent of osteomyelitis, the efficacy of therapy, and bone growth. Radiographs were visually assessed and graded on a scale of 0 to 4: 0, normal, no difference between left and right tibia; 1, elevation and/or disruption of the periosteum, soft-tissue swelling; 2, disruption of ≤10% of normal bone architecture; 3, disruption of 10%-40% of normal bone architecture; and 4, disruption of >40% of normal bone architecture.[5]

### Killing of the animals

After clinical and radiographic examination, rats in groups 1-2 were killed 21 days after the induction surgery, and rats in groups 3-5 were killed 21 days after the debridement surgery, by using excessive dose of thiopental (200 mg/kg).[21] After killing the animals, left tibias were removed for histopathologic and bacteriologic examination.

### Histopathologic examination

Tissue samples were fixed in 10% buffered formalin and decalcified in a working solution of hydrochloric acid/formic acid 20 times greater than their own volume. Solutions were changed daily until decalcification was complete (96 h). After decalcification, specimens

were thoroughly washed under running tap water and embedded in paraffin. Specimens were sectioned into 5-μm slices, placed on polylysine-coated slides, and stained with hematoxylin and eosin. Slides were randomly examined under a light microscope (Olympus BX51; Olympus Corp., Tokyo, Japan) at ×40 magnification by a pathologist blinded to the specimen groups.

The disease severity was assessed according to Smeltzer et al.[22] by evaluating intraosseous acute inflammation (IAI), intraosseous chronic inflammation (ICI), periosteal inflammation (PI) and bone necrosis (BN). The scoring for IAI was: 0, not present; 1, minimal to mild inflammation with no intramedullary abscess; 2, moderate to severe inflammation with no intramedullary abscess; 3, minimal to mild inflammation with intramedullary abscess; and 4, moderate to severe inflammation with intramedullary abscess. For ICI, the scoring was: 0, not present; 1, minimal to mild chronic inflammation with no significant intramedullary fibrosis; 2, moderate to severe chronic inflammation with no significant intramedullary fibrosis; 3, minimal to mild chronic inflammation with significant intramedullary fibrosis; and 4, moderate to severe chronic inflammation with significant intramedullary fibrosis. For PI, the scoring was: 0, not present; 1, minimal to mild inflammation with no subperiosteal abscess formation; 2, moderate to severe inflammation with no subperiosteal abscess formation; 3, minimal to mild inflammation with subperiosteal abscess formation; and 4, moderate to severe inflammation with subperiosteal abscess formation. And for BN, the scoring was: 0, no evidence of necrosis; 1, single focus of necrosis without sequestrum formation; 2, multiple foci of necrosis without sequestrum formation; 3, single focus of sequestrum; and 4, multiple foci of sequestrum.

A total Smeltzer score (SS) of ≥4 out of a maximum of 16 was considered to be positive for osteomyelitis. Scores for individual parameters were also analyzed separately to better determine the effect of tigecycline-impregnated CHA on osteomyelitis.

### Microbiologic examination

Bone specimens were taken from all animals after they were killed and during debridement surgery under aseptic conditions. All microbiological analyses were conducted by a single microbiologist blinded to the specimen groups. Specimens were placed in sterile tubes and weighed using a sensitive scale. Ten milliliters of saline solution was added to each specimen, which was mechanically homogenized inside a sterile bag using a stomacher. Specimens were serially diluted and prepared using the tryptic soy agar plating technique.

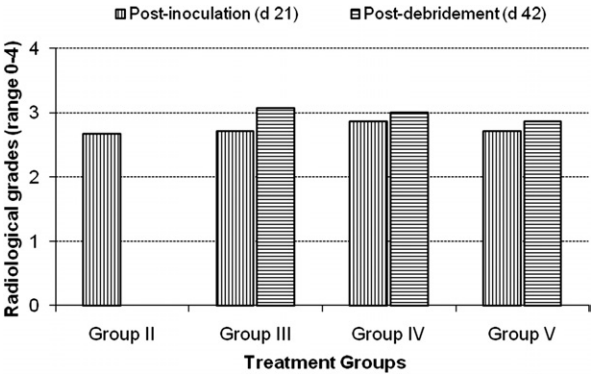



Fig. 2. Radiographic osteomyelitis scoring per Mader et al.[5] was used to analyze disease severity 21 days after inoculation and 21 days after debridement. The maximum score of 4 reflects severe chronic osteomyelitis in the entire tibia with >40% disruption of normal bone architecture. Numerically greater disease severity scores on day 21 than on day 42 were insignificant ($P > .05$).

Slides were incubated for 24 hours at 37°C, after which bacteria were identified and enumerated (CFU/g bone).

### Statistical analyses

Statistical analyses were performed by using Mann-Whitney *U* tests (SPSS, version 17.0, Chicago, IL) to identify differences in histopathologic, radiologic, and microbiologic findings between groups, with a level of $P < .05$ regarded to be statistically significant. Bacteria counts were subjected to log transformation for normalization prior to statistical analysis.

## RESULTS

### Macroscopy

Redness, swelling, sinus formation, and pus discharge were observed in the affected region ~2 days after osteomyelitis induction in groups 2-5 (Fig. 1, *A*). The animals continued to be ambulatory, although limping and tibial deformation were observed. On day 21 after debridement surgery, with the exception of tibial deformation, these findings were no longer observed in rats in group 5.

### Radiography

X-ray evaluation performed on day 21 after inoculation revealed that changes attributed to the osteomyelitic process consisted mainly of soft tissue swelling, bone lucency, diaphyseal deformation and widening, periosteal reaction (thickening and/or elevation), and osteolysis in the cortex in the affected tibia (Fig. 1, *B*). Postinoculation (day 21) and postdebridement (day 42) radiographs revealed no significant differences in osteomyelitis among groups 2-5 (Fig. 2).

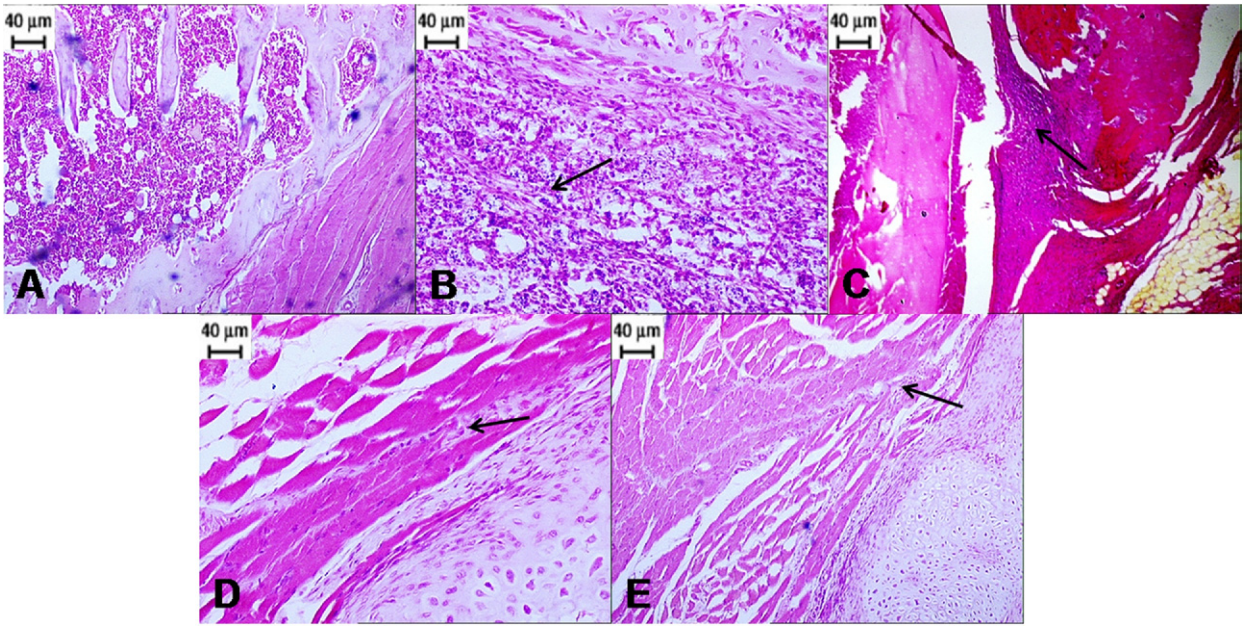



Fig. 3. Microscopic examination of a rat left hind tibia. Hematoxylin and eosin, ×100. **A,** Group 1 [sham group (n = 5)]: diaphyseal region in the left tibia is normal. **B,** Group 2 [negative control, nontreated infected group (n = 6)]: multiple abscesses can be seen in the intramedullary stromal tissue within the diaphyseal region. **C,** Group 3 [debridement surgery alone (n = 7)]: there are relatively few inflammatory cells compared with the negative control group in the intramedullary stromal tissue within the diaphyseal region. **D,** Group 4 [debridement surgery plus CHA material without antibiotic impregnation (n = 7)]: there are relatively few inflammatory cells compared with the negative control group in the intramedullary stromal tissue within the diaphyseal region. **E,** Group 5 [debridement surgery plus tigecycline-impregnated CHA material (n = 7)]: there are a few inflammatory cells in diaphyseal region.

## Histopathology

In group 2, histopathologic examination revealed the presence of macrophages, degenerated cells, necrotic cells, bone necrosis, dense polymorphonucleated leukocytes (PNL), apoptotic cells, vascular congestion, periosteal inflammation, a low level of plasma and lymphocytes, and vascular proliferation. In groups 3-4, there was a decline in vascular congestion and PNL numbers and an increase in plasma, lymphocyte and macrophage levels. Vascular congestion and leukocyte levels were lowest and vascular proliferation highest in group 5 (Fig. 3).

Compared with group 1, significant increases in IAI ($P = .003$), PI ($P = .003$), BN ($P = .003$), and SS ($P = .004$) scores in group 2 revealed success of the osteomyelitis induction (Table I). All treatment approaches significantly alleviated histopathologic parameters compared to group 2. There were 41%, 41%, and 85% declines in IAI in groups 3, 4, and 5, respectively ($P = .002$). The ICI scores increased by 247% and 171% in groups 3 and 4 ($P = .017$; $P = .038$; respectively), whereas it decreased by 14% in group 5 ($P = .128$). In groups 3 and 4, the PI score decreased by 69% ($P = .001$) and in group 5 it decreased by a much greater extent (81%; $P = .002$). The BN score also decreased by 65% in groups 3 and 4 ($P = .002$), and BN totally diminished in group 5. Overall, compared with group 2, total SS scores declined by 37% in group 3 ($P = .004$), 44% in group 4 ($P = .002$), and 83% in group 5 ($P = .003$). Total SS scores did not differ significantly between groups 3 and 4 ($P = .165$); however, the difference in total SS scores between groups 4 and 5 was statistically significant ($P = .002$). Despite statistical difference in SS scores between groups 1 and 5 ($P = .003$), the value in group 5 was fewer than 4.00 (Table I).

## Microbiology

Osteomyelitis was also reflected by the highest bacteria count ($5 \times 10^5$ CFU/g bone) in group 2 (Table I). The mean bacteria counts in groups 3, 4, and 5 were $1.6 \times 10^4$, $9.7 \times 10^3$, and $2.9 \times 10$ CFU/g bone, respectively (Table I). Compared with group 2, decrease in bacterial count was 26% in group 3 ($P = .01$), 29% in group 4 ($P = .008$), and 79% in group 5 ($P = .003$; Table I). Although infection was completely eradicated in 2 rats in group 5, the mean bacteria count for group 5 was statistically higher than that of group 1 ($P = .024$).

**Table I.** Effects of debridement surgery, calcium hydroxyapatite and tigecycline-impregnated calcium hydroxyapatite in the treatment of chronic osteomyelitis*

| | *HE*† | | | | | *ME*‡ |
|---|---|---|---|---|---|---|
| *Group** | *IAI* | *ICI* | *PI* | *BN* | *SS* | *Log (BC)* |
| 1 | $0.00 \pm 0.00^{d}$ | $0.00 \pm 0.00^{d}$ | $0.00 \pm 0.00^{d}$ | $0.00 \pm 0.00^{c}$ | $0.00 \pm 0.00^{d}$ | $0^{d}$ |
| 2 | $3.50 \pm 0.30^{a}$ | $1.00 \pm 0.51^{c}$ | $3.67 \pm 0.29^{a}$ | $3.67 \pm 0.29^{a}$ | $11.83 \pm 0.66^{a}$ | $5.70^{a}$ |
| 3 | $1.43 \pm 0.28^{b}$ | $3.57 \pm 0.28^{a}$ | $1.14 \pm 0.23^{b}$ | $1.29 \pm 0.26^{b}$ | $7.43 \pm 0.40^{b}$ | $4.22^{b}$ |
| 4 | $1.43 \pm 0.28^{b}$ | $2.71 \pm 0.26^{b}$ | $1.14 \pm 0.23^{b}$ | $1.29 \pm 0.26^{b}$ | $6.57 \pm 0.37^{b}$ | $4.03^{b}$ |
| 5 | $0.71 \pm 0.28^{c}$ | $0.86 \pm 0.23^{c}$ | $0.71 \pm 0.30^{c}$ | $0.00 \pm 0.00^{c}$ | $2.03 \pm 0.43^{c}$ | $1.18^{c}$ |

Histopathologic and microbiologic values are least square means ± standard error.
[a,b,c,d]Different superscripts within the same columns indicate significant difference ($P < .05$).
*Group 1, sham; group 2, nontreated infected; group 3, debridement surgery; group 4, debridement plus calcium hydroxyapatite (CHA); group 5, debridement plus tigecycline-impregnated CHA.
†*HE*, Histopathologic evaluation: histopathological data scored according to Smeltzer et al.[22] The maximum possible score is 4 for IAI, ICI, PI, and BN and 16 for SS. *IAI,* Intraosseous acute inflammation; *ICI*, intraosseous chronic inflammation; *PI*, periosteal inflammation; *BN*, bone necrosis; *SS*, total Smeltzer score.
‡*ME*, Microbiologic evaluation: *BC*, bacteria count (CFU/g bone).

## DISCUSSION

Antibiotic treatment of osteomyelitis continues to be challenging and varies by countries and resistance status.[23] Infections caused by MRSA are difficult to treat and often have a poor prognosis.[24] For years, the treatment of serious MRSA infections has been based on the use of glycopeptides, vancomycin, and teicoplanin, but increasing rates of heteroresistance and glycopeptide tolerance have encouraged the search for newer agents.[4] Tigecycline was the first of a new class of antimicrobial agents, glycylcyclines, to become commercially available. Tigecycline has demonstrated promising activity against clinical MRSA isolates.[25] Verkade et al.[26] reported that tigecycline displayed good activity against MRSA strains in vitro.

The number of reports dealing with the efficacy of tigecycline in the treatment of osteomyelitis is limited. Kandemir et al.[27] compared the effects of tigecycline and teicoplanin in the treatment of osteomyelitis of MRSA origin and reported that tigecycline administered subcutaneously might be a suitable pharmacologic alternative to teicoplanin. Yin et al.[28] reported an infection clearance level of 90% with a subcutaneous injection of tigecycline used to treat osteomyelitis of MRSA origin in a rabbit model.

Treatment of osteomyelitis usually consists of radical surgical debridement of infected tissue, refilling of the dead space, and soft tissue coverage, followed by 4-6 weeks of intravenous antimicrobial therapy.[29,30] Although both hydroxyapatite and PMMA may be used to fill the dead space left by debridement, hydroxyapatite is the more porous of the 2 materials, which allows higher drug concentrations to be delivered over a long period of time,[31] thereby maintaining local antibiotic levels much higher than the minimum level of bactericidal concentration.[4,15] In addition, the gradual resorption of CHA and its replacement with newly formed bone, which usually occurs within a few months, makes it superior to materials such as PMMA that are not resorbed and therefore require surgical removal.[15]

Bacteria counts for groups 3 and 4 were similar, and both were lower compared with group 2 (Table I). Moreover, histopathologic analysis showed no differences in intramedullary abscesses between groups 3 and 4. However, the mean total SS scores were >4 in groups 3 and 4 (Table I). No improvement in microbacterial and histopathologic parameters observed in group 4 may be ascribed to the fact that whatever bacteria remained after debridement constituted a good adhesion surface for the implant material.[32]

An in vivo study in a rat model conducted by Korkusuz et al.[33] found that antibiotic-impregnated CHA ceramic composites eliminated osteomyelitis within 3 weeks of implantation. However, numerous other studies studying various implants and antibiotics used to locally treat infection have shown reductions in numbers of bacteria but not complete eradication.[5,15,34-38] In our study, the failure to completely eradicate osteomyelitis may be ascribed to the short treatment time and to the use of locally administered antibiotics without complementary systemic treatment. Nonetheless, histologic indicators showed significant improvements (Table I).

Radiographically, soft tissue swelling and periosteal thickening and/or elevation represent the earliest signs of osteomyelitis. These are followed by lytic changes within the bone matrix related to subacute or chronic osteomyelitis.[39] In the present study, radiologic examination showed clear changes within the bone due to infection and helped to confirm the development of chronic osteomyelitis. According to Butt,[39] even in patients who receive appropriate antimicrobial treatment, radiographic evidence of healing may emerge later than clinical evidence. In the present study, compared with 21 days after induction, although no radio-

graphical improvement (Fig. 2) was observed 42 days after induction, both histopathologic and microbiologic evaluation (Table I) showed a great reduction in infection.

In conclusion, tigecycline-impregnated CHA is potentially useful in the local treatment of chronic osteomyelitis of MRSA origin. Further preclinical investigations on the use of tigecycline with CHA for the treatment of osteomyelitis in human and veterinary medicine are warranted.

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*Reprint requests:*

Dr. Mahir Kaya
Department of Surgery
Faculty of Veterinary Medicine
Atatürk University
25240, Erzurum
Turkey
kaya25mali@hotmail.com