Prophylaxis of implant-related staphylococcal infections using tobramycin-containing bone cement

Marc W. Nijhof,[1] Wouter J. A. Dhert,[1] André Fleer,[2] H. Charles Vogely,[1] Abraham J. Verbout[1]
[1]*University Cluster of Orthopaedics, University Medical Center Utrecht, G05.228, P.O. Box 85500, NL 3508 GA Utrecht, The Netherlands*
[2]*Department of Medical Microbiology, University Children's Hospital and Eijkman–Winkler Institute, University Medical Center Utrecht, Utrecht, The Netherlands*

Received 20 August 1999; revised 14 April 2000; accepted 14 April 2000

Abstract: In a rabbit model, premixed tobramycin-containing bone cement was studied for its efficacy to prevent infections with two frequently encountered staphylococcal species in arthroplasty surgery. After intramedullary inoculation with staphylococci, either standard or premixed tobramycin-containing Simplex-P bone cement was injected in the right femur of 120 rabbits. Development of infection was examined by culture of femoral bone after 7 or 28 days. Loss of body weight and elevated erythrocyte sedimentation rate in the control rabbits inoculated with *Staphylococcus aureus* were seen in the first postoperative week, returning to normal in 28 days. Inoculation with *Staphylococcus epidermidis* resulted only in a low-grade infection. All rabbits receiving premixed tobramycin-containing bone cement were free of signs of infection, and all their cultures were negative. Culture yield from *Staphylococcus aureus* controls increased with time and inoculum dose. *Staphylococcus epidermidis* controls needed higher inoculum doses to establish an infection, while culture yield decreased in time. These differences in mode of prosthesis-related infection are explained by differences in virulence factors. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res, 52, 754–761, 2000.

Key words: tobramycin; bone cement; prophylaxis of infection; animal model; staphylococci

INTRODUCTION

Staphylococcus aureus and *Staphylococcus epidermidis* are the most frequently found organisms involved in arthroplasty-related infection. Incidence rates after total joint arthroplasty of *Staphylococcus epidermidis* (26–38% of infections) are somewhat higher than those for *Staphylococcus aureus* (16–24% of infections) and have increased in the last decade.[1–5] Apparently, staphylococci have surface properties that enable them to adhere and grow on implant-surfaces. Some of these surface factors that mediate adhesion to biomaterials have been more or less well characterized for *Staphylococcus epidermidis*.[6] This surface-adherent growth mode might even hamper killing of the bacteria by antibiotics.[7] Darouiche et al. (1994) showed that *Staphylococcus epidermidis*, grown on stainless steel nuts in the presence of vancomycin could not be eradicated completely even though high levels of vancomycin were reached in the biofilm.[8]

The use of antibiotic-containing bone cement in arthroplasty surgery provides for high concentrations of antibiotics at the implant side, while the low systemic levels of antibiotics reduce possible systemic toxicity such as in the case of aminoglycosides. Among the latter class of antibiotics, tobramycin is one of the prime candidates for the preparation of antibiotic bone cement because of its antibacterial spectrum. This spectrum includes staphylococci as well as aerobic Gram-negative bacteria like *Pseudomonas aeruginosa*, which is an example of another isolate involved in arthroplasty-related infections.[5,9] As compared to gentamicin, tobramycin has a greater activity against *Pseudomonas aeruginosa*.[10–12] In numerous hospitals, especially in the United States, antibiotic-containing bone cement is prepared by adding tobramycin powder perioperatively to bone cement and subsequent hand-mixing.[13] This hand-mixing of antibiotics with bone cement does not allow for a consistent and controllable quality of cement. A standardized prepara-

Correspondence to: Marc W. Nijhof, M.D., University Cluster of Orthopaedics, University Medical Center Utrecht, G05.228, P.O. Box 85500, NL 3508 GA Utrecht, The Netherlands (e-mail: m.nijhof@chir.azu.nl)

No benefit of any kind will be received either directly or indirectly by the authors.

tion of antibiotic-containing bone cement benefits not only future infection-based efficacy studies, but also the evaluation of its biomechanical properties. Although adding low quantities (approximately 1 g per unit) of tobramycin has minimal effects on the fatigue life of cement, amounts of more than 2 g of antibiotics per unit of cement has been shown to decrease the strength of the cement.[14,15]

The efficacy of premixed tobramycin-containing bone cement in preventing implant infections has not been investigated before. In a previous study on the release of tobramycin from commercially prepared tobramycin-containing bone cement, the minimal inhibitory concentrations of this antibiotic against staphylococci were exceeded for up to 28 days.[16] The purpose of the present study was to investigate the efficacy of the same bone cement in the prevention of local *Staphylococcus aureus* and *Staphylococcus epidermidis* infections.

MATERIALS AND METHODS

Experimental design

Premixed tobramycin-containing bone cement or plain bone cement (controls) was introduced into the medullary canal of the rabbits' right femur. Prior to cement injection, this medullary canal had been inoculated with *Staphylococcus aureus* or *Staphylococcus epidermidis* in five increasing doses. To monitor infection, the amount of bacteria in the cortex of the femur was quantified after 7 and 28 days, when the animals were killed. In both bacteria groups, 40 rabbits (20 tobramycin-cement, 20 controls) were killed after 7 days, and 20 rabbits (10 tobramycin-cement, 10 controls) after 28 days.

The guidelines according to the Dutch act on animal experiments (1985) have been observed.

Animals

120 Healthy adult female New Zealand White rabbits (Ico: NZW, mean weight 3.1 kg, range 2.6–3.6 kg) were obtained one week prior to surgery to acclimatize them to the housing in the Central Animal Laboratory Institute, Utrecht University. The animals were fed with 80–100 g antibiotics-free rabbit diet and water *ad libitum.*

Cement

Surgical Simplex® P bone cement, premixed with 1.0 g tobramycin as a sulphate in 60 g methyl methacrylate (batch: N6043), was supplied by Howmedica Inc., Rutherford, NJ. This radiopaque bone cement is a mixture of a liquid component (monomer) and a powder component (polymer), both sterilized. The composition of the powder component is: polymethyl methacrylate (6.0 g), methyl methacrylate-styrene copolymer (30 g), barium sulphate (4.0 g), and tobramycin (as a sulphate, 1.0 g). The liquid component (20 mL) is composed of methyl methacrylate (97.4% vol/vol), *N,N*-dimethyl-p-toluidine (2.6% vol/vol) and hydroquinone (75 ± 15 parts per million). The shelf-life of premixed tobramycin-containing bone cement is 2 years. In this study, all cement was used within 18 months after manufacture and stored at room temperature until use.

Surgical Simplex® P bone cement without antibiotics was used as control (Howmedica, Inc).

Bacterial strain

Two bacteria were used to contaminate the femur: *Staphylococcus aureus,* strain Wood-46 (American Type Culture Collection Number 10832) and *Staphylococcus epidermidis,* strain O-47.[17] The inoculum sizes of *Staphylococcus aureus* were 10^3, 10^4, 10^5, 10^6, and 10^7 colony-forming units (CFU); those of *Staphylococcus epidermidis* were 10^8, $10^{8.5}$, 10^9, $10^{9.5}$, and 10^{10} CFU. To prepare the different inocula, the concentration of bacteria in Mueller–Hinton broth had been determined by serial dilution and plating on blood agar. The injection volume of each inoculum was 0.1 mL. Using the E-test, we determined the minimal inhibitory concentration (MIC) of tobramycin to be 0.125 and 0.064 μg/mL, for *Staphylococcus aureus* Wood-46 and *Staphylococcus epidermidis* O-47, respectively.[18]

Anesthesia

Surgery was performed under strict aseptic conditions and under general inhalation anesthesia. Preoperatively the rabbits were weighed. The anesthesia was prepared by an intramuscular injection of 4 mg methadone, 4 mg acepromazinemaleate, and 0.5 mg atropine. Blood samples on leukocyte counts and erythrocyte sedimentation rate were taken of all rabbits preoperatively. A pressure line was introduced into the auricular artery for measuring blood pressure. Subsequently, the anesthesia was induced by an intravenous injection of 8–12 mg etomidate. An endotracheal tube (#3) was introduced, through which the anesthesia was maintained by a 1:1 mixture of nitrous oxide, oxygen, and halothane 1%.

Postoperatively, pain relief was provided by 3 mg nalbufine intramuscularly immediately postoperative and subsequently 0.09 mg buprenorfine intramuscularly (if necessary, buprenorfine injection was repeated postoperatively).

Operative technique

The skin of the right leg was clipped, and the rabbit was placed with its left side on the table. The operative area was

disinfected with povidone-iodine and isolated by sterile drapes. Subsequently, a skin incision (approximately 3 cm) was made over the trochanter tertius of the right femur, parallel to the femoral shaft. The trochanter tertius was exposed by splitting the fascia, retracting the femoral biceps and coccygeofemoral muscles postero-medially, and scraping the periost. Using an air-pressured AO mini-drill, the cortex was penetrated by a small drill (diameter 1.2 mm). Subsequently, this hole was widened and the femoral canal was reamed with 50-mm drills and fraises up to 4 mm in width until a silicon tube (monitor line, outer diameter 3 mm) could be inserted. Cooled PMMA bone cement (4°C) was vacuum-mixed (Simplex® cement vacuum-mixer, Howmedica Inc., Rutherford, NJ) (0.9 bar) for 60 s (tobramycin-containing bone cement) or 100 s (plain bone cement) on the surgical table. Subsequently, a 6-mL syringe with a 2.0 cm long silicon tube (outer diameter 3.0 mm, inner diameter 1.5 mm) was filled with cement, weighed, and placed in an adapted device on an applicator gun. During these steps, the medullary canal was washed with sterile physiologic saline, suctioned, and inoculated with bacteria. Immediately after inoculation, approximately 1.2 mL of the doughy cement was injected gently into the femoral canal, while the syringe was slowly being retracted. The expansion of the cement sealed off the entrance of the medullary canal at this moment, thereby preventing any spill of the bacterial suspension out of the medullary canal. The cement was allowed to polymerize *in situ*. The syringe was weighed again to determine the amount of cement injected (mean weight of cement in all rabbits was 1.24 ± 0.16 gram). After wound drainage with sterile saline solution, the fascia, subcutis, and cutis were closed with Vicryl® 3-0.

Follow-up

Postoperatively, localization of the cement was evaluated by routine anteroposterior and lateral radiographs of the right femur (Fig. 1), and the rabbits were allowed to recover in a temperature-controlled recovery cage.

The rabbits were monitored by a daily clinical examination, with special attention to wound healing, the presence of a fracture, eating, activity level, and body temperature.

Erythrocyte sedimentation rate in the first hour and leukocyte counts were measured preoperatively and weekly postoperatively.

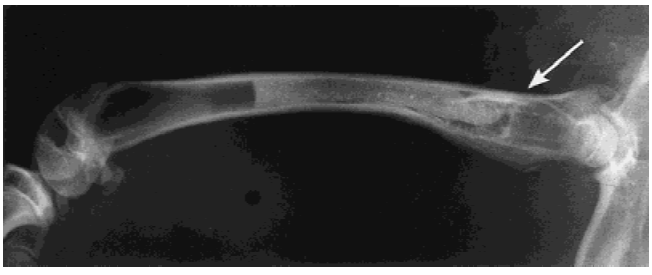

Figure 1. Lateral radiograph of the right femur of a rabbit, which shows the injected cement *in situ*. The arrow indicates the site of injection of the cement.

At day 7 or 28, rabbits were killed with an overdose of pentobarbital sodium intravenously.

Post mortem sample acquisition

Excision of the femora was performed under strict aseptic conditions. The left femur (no bacteria, no cement) was excised to study contamination during the culture procedure. The skin of the hind limbs was clipped and, with the rabbit placed in prone position on the table, disinfected with a 2% tincture of iodine and isolated by sterile drapes. The left (control) and right femur were excised from all animals and tissue debris was cleaned off. First a bone fragment was taken from the left femur from a region corresponding to that of the right femur. Then, using a high-speed circular saw, the external surface of the right femur was notched circumferentially at each end of the cement plug and longitudinally on two sides, posterior and anterior. An osteotome was used to free the lateral half of the bone from the medial half. Without damaging the cement plug, the cement was carefully separated from the bone in order to prevent release of tobramycin, which could influence the sampling of bacteria.[19] Only full-thickness cortex adjacent to the cement was used for both bacteriologic culture and histology; bone that was not directly adjacent to the cement was discarded.

Bacteriological culture

All the bone fragments of the lateral half of each femur (in each case approximately 1 g) were ground in a sterile metal mortar. The samples were homogenized in 10 mL of phosphate buffered saline (pH 7.4) using a tissue grinder (Polytron PT 3100 tissue grinder, Kinetica Benelux BV, Best, The Netherlands), 3 min at 2500 rounds per min and subsequently for 5 min at 6000 rounds per min.

A volume of 10 μL of the homogenate (containing 1.0 mg of bone) was then plated on blood agar plates in serial 10-fold dilutions. After an overnight incubation at 37°C, the samples were counted for viable bacteria. For each femur, the number of viable bacteria per gram of bone was calculated (minimum 1000 CFU per gram of bone). Infection was defined as a positive culture.

Histology

The medial half of the bone adjacent to the bone cement was used for histological evaluation and fixed in 4% buffered formalin. After decalcification and dehydration, the cortex was embedded in paraffin and sectioned on a mictotome (Reichert–Jung 2030, Biocut, Leica, Rijswijk, The Netherlands). The sections were mounted on slides and stained with hematoxylin and eosin.

Statistical analysis

The results of the bacteriological cultures were analyzed using the SPSS (Statistical Package for the Social Sciences)

version 6.1 for the Macintosh. Differences were considered significant at $p \leq 0.05$. Animals with a comparable history were grouped. To each group one of the five inoculum doses and one of the two follow-up periods were assigned. One half of each group received tobramycin, the other half not, which formed the controls. For each animal a response of the 0/1 type was recorded (negative or positive culture) and the number of cultured bacteria. In the first case, a Mantel–Haenszel type of analysis is called for with a test for trend over the strata (inoculum doses), which was carried out using logistic regression. Differences in response over the strata may be caused by differences between groups. It is, however, unlikely that this would be exactly like a trend, which is more plausibly a dose effect. In the second case, a two-way analysis of variance was used to investigate the effect of follow-up and inoculum dose. In particular, a positive relationship of the inoculum dose with the number of cultured bacteria was analyzed by means of polynomial contrasts.

RESULTS

General

In the *Staphylococcus aureus* group, one rabbit with tobramycin-containing bone cement (7-days follow-up group, inoculum 10^5 CFU) and one control (10^7 CFU, 28-days follow-up group) died just after the cementing procedure. The controls showed a loss of body weight and elevated erythrocyte sedimentation rate at 7 days. At 28 days, these parameters had returned to normal, but the macroscopical aspect of the right femur of these rabbits was suggestive of a local osteomyelitic process (thickened and soft cortex, pus). The *Staphylococcus epidermidis* controls, as well as all rabbits receiving tobramycin-cement recovered well from surgery and did not develop clinical signs of infection (erythrocyte sedimentation rate, leukocyte count, body temperature, and body weight remained normal).

Bacteriological cultures

No positive cultures were found in any of the rabbits receiving tobramycin-cement [Figs. 2(a) and 2(b)]. Twenty-seven out of 29 of the *Staphylococcus aureus* controls and 16 out of 30 of the *Staphylococcus epidermidis* controls had positive bacterial cultures. One control rabbit in the 7-day follow-up group that had received 10^7 CFU of *Staphylococcus aureus* was killed after 5 days, because it was severely ill. The results of the culture of its right femur (^{10}log 5.89) was included as a 7-day result. Table I shows an overview of the incidence of infection in the various groups. For both staphylococcal species, the prophylactic effect of tobramycin-containing bone cement was significant ($p < 0.001$). Because all tobramycin-treated animals (for both follow-up periods and both bacteria species) did not show positive cultures, differences in trend of the inoculum dose between controls and tobramycin-treated animals have not been statistically tested. The number of cultured bacteria in the *Staphylococcus aureus* controls increased significantly from 7 to 28 days follow-up ($p = 0.044$, $F_{1,19} = 4.64$). Testing for a linear relationship [trend of a positive relationship of inocu-

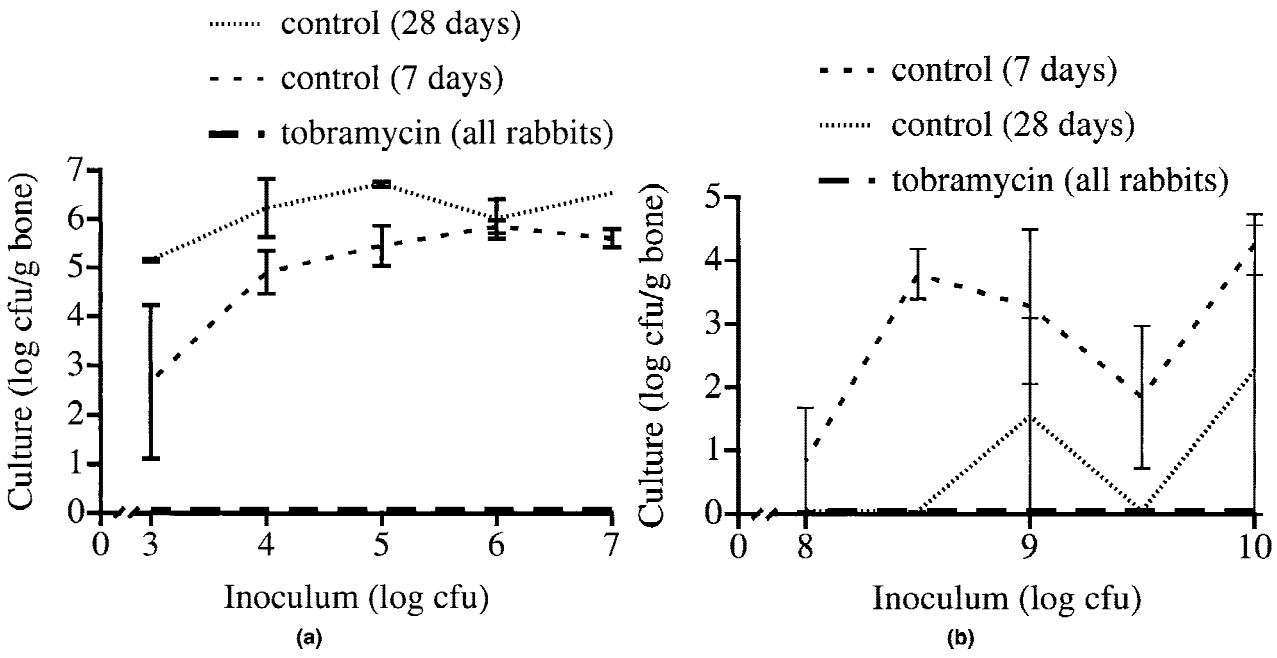

Figure 2. (a) Results of cultures (mean ± SEM) of rabbits contaminated with *Staphylococcus aureus.* (b) Results of cultures (mean ± SEM) of rabbits contaminated with *Staphylococcus epidermidis.*

TABLE I(a)
Incidence of Infections in the *Staphylococcus aureus* Groups

Inoculum (^{10}log CFU)	7 days		28 days	
	Tobramycin	Control	Tobramycin	Control
3	0/4	2/4	0/2	2/2
4	0/4	4/4	0/2	2/2
5	0/3	4/4	0/2	2/2
6	0/4	4/4	0/2	2/2
7	0/4	4/4	0/2	1/1
Total	0/19	18/20 (90%)	0/10	9/9 (100%)

TABLE I(b)
Incidence of Infections in the *Staphylococcus epidermidis* Groups

Inoculum (^{10}log CFU)	7 days		28 days	
	Tobramycin	Control	Tobramycin	Control
8	0/4	1/4	0/2	0/2
8.5	0/4	4/4	0/2	0/2
9	0/4	3/4	0/2	1/2
9.5	0/4	2/4	0/2	0/2
10	0/4	4/4	0/2	1/2
Total	0/20	14/20 (70%)	0/10	2/10 (20%)

lum with the extent of infection, see Fig. 2(a)] showed a significant difference of inoculum size ($p = 0.034$, one sided). For the *Staphylococcus epidermidis* controls, a significantly lower number of bacteria was cultured after 28 days as compared to 7 days ($p = 0.07$, $F_{1,20} = 9.06$). The effect of the inoculum concentration (linear relationship) was not significant ($p = 0.082$).

All cultures from left femora (not operated on) were negative.

Histology

In the *Staphylococcus aureus* group, the majority of rabbits in the control group showed a moderate periosteal reaction of the femoral cortex next to the cement after 7 days [Fig. 3(a)]. At 28 days follow-up, the *Staphylococcus aureus* controls showed, in addition to a more severe periosteal reaction, other signs for infection such as destruction of the cortex, enlarged Haversian canals, and a leukocytes infiltrate [Fig. 3(b)]. These changes were also present in the *Staphylococcus epidermidis* controls, but were less pronounced. We found no histological signs of infection in any of the rabbits that received tobramycin-containing bone cement (Fig. 4).

DISCUSSION

In the present study, we showed that premixed tobramycin-containing bone cement did prevent infection after inoculation of *Staphylococcus aureus* and *Staphylococcus epidermidis.* These findings support our previous study, which showed that the release of tobramycin from this cement, 28 days after insertion, is still more than 40 times the minimal inhibitory concentrations for these staphylococci.[16] However, tests of susceptibility based on minimal inhibitory concentration may not be accurate for biomaterial-adherent bacteria and, therefore, *in vivo* studies are required.[7] Previously, other infection studies in the rabbit, rat, or dog have shown the efficacy of bone cements containing gentamicin or erythromycin/colistin.[20–23] The efficacy of these antibiotic-bone cement combinations is less clear in the clinical setting: Lynch et al. found the efficacy of the gentamicin loaded cement to be statistically proven only in revision operations, not in primary arthroplasties.[24] Josefsson et al. compared the prophylactic efficacy of systemic antibiotics vs. gentamicin bone cement. The difference in favor of the antibiotic bone cement group had lost its statistical difference at 10 years follow-up.[25,26] Results from the Norwegian arthroplasty show that the additional use of antibiotic bone cement can reduce the infection rate compared to systemic antibiotic only.[27]

The advantage of antibiotic-containing bone cement is the high local release of antibiotic, which can overcome the decreased susceptibility of bacteria growing on an infected prosthesis. High local release of aminoglycosides like tobramycin increases the rate of bacterial killing because of their concentration-dependent antibacterial activity.[28] Eckman et al. found effective wound and serum levels of tobramycin in patients with compound fractures after prophylactic treatment with tobramycin-impregnated PMMA beads.[29] No acute wound infections were seen in these 70 patients. Lyons et al. showed that tobramycin-PMMA beads prevented adherence of *Staphylococcus*

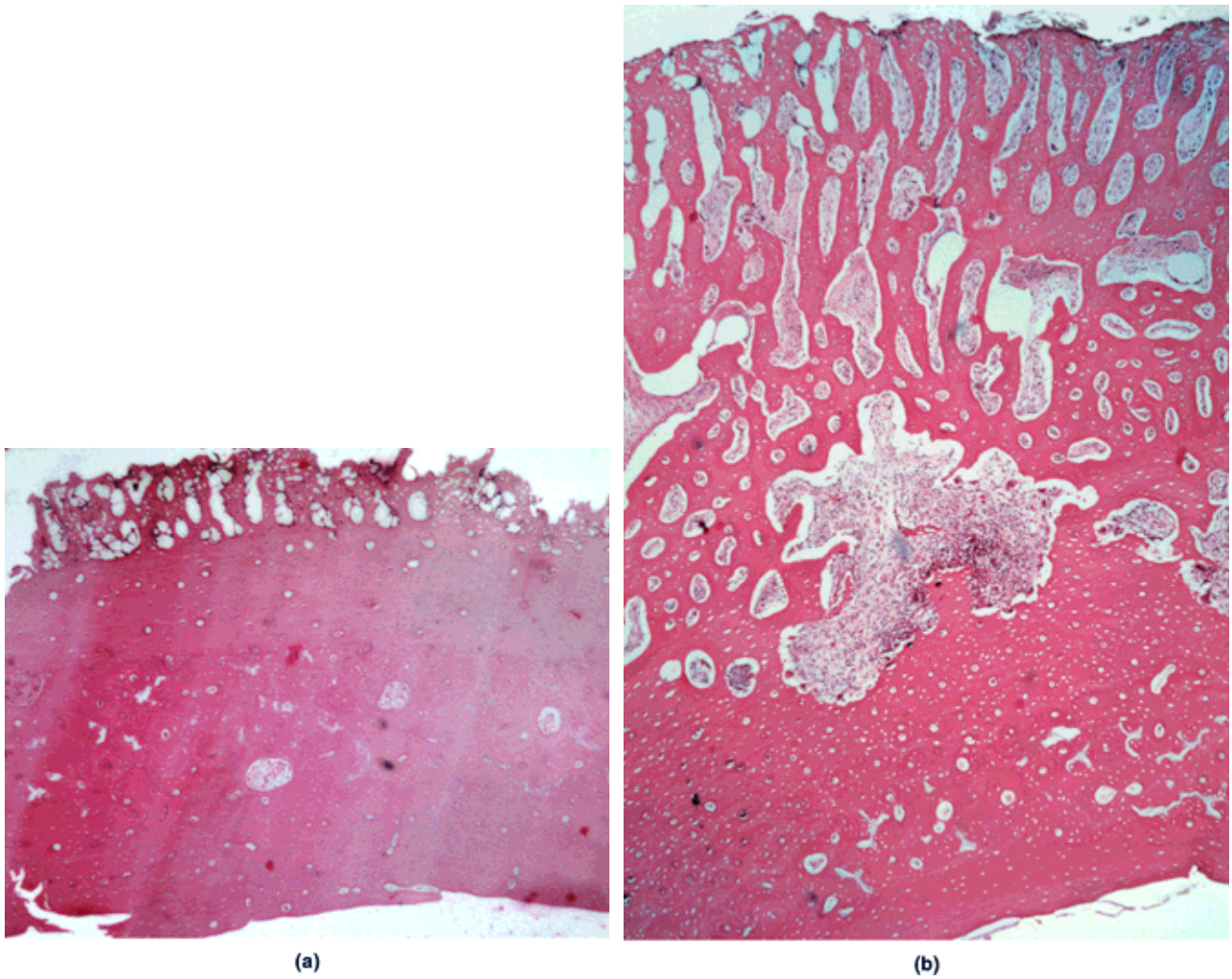

Figure 3. Photomicrographs (hematoxylin and eosin, ×50) of tissue sections of control rabbits contaminated with *Staphylococcus aureus* showing (a) minimal periosteal reaction after 7 days, and (b) severe periosteal thickening, destruction of cortex, leukocyte infiltrate, and granulation tissue after 28 days.

aureus in a rabbit model of a contaminated fracture.[30] The release from hand-mixed tobramycin bone cement has been investigated in primary total hip arthroplasties, but these studies did not mention whether infections were actually prevented.[31,32] In another study, infection recurred in one out of ten patients, treated with revision total hip arthroplasty using bone cement hand-mixed with tobramycin (0.5 g tobramycin per unit cement).[33] Perioperatively, no systemic antibiotic had been given in this patient, who had intraoperative positive cultures for *Pseudomonas aeruginosa*. Hofmann et al. treated 26 infected total knee arthroplasties using hand-mixed tobramycin bone cement (both for the spacer and fixation of the revision prosthesis) together with systemic antibiotics.[34] After a mean follow-up of 30 months, no infection recurred.

The *Staphylococcus aureus* controls showed an inoculum-size dependent infection, which increased up to 28 days follow-up. In contrast, the virulence of *Staph-*

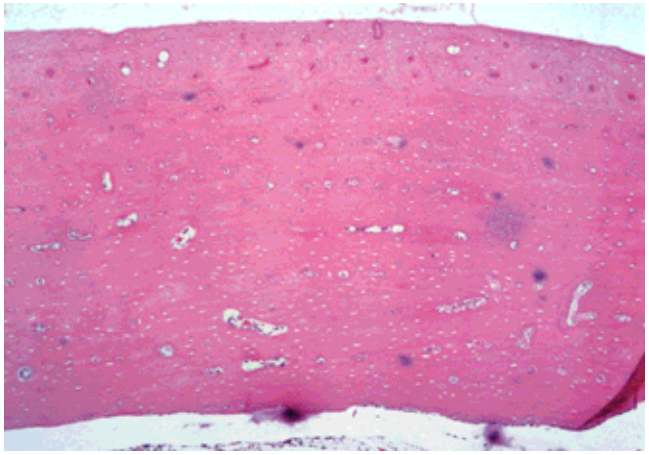

Figure 4. Photomicrograph (hematoxylin and eosin, ×50) of a tissue section from a rabbit with tobramycin-containing bone cement contaminated with *Staphylococcus aureus*, showing no signs of infection after 28 days.

ylococcus epidermidis appeared to be lower, given the higher inoculum dose needed to establish an infection and the lower incidence of infection. *Staphylococcus aureus* produces a number of extracellular and cell-associated virulence factors such as enterotoxins, collagen- and fibronectin-specific receptors, and exopolysaccharides, and probably, therefore, causes more evident signs of infection than *Staphylococcus epidermidis*.[35] *Staphylococcus epidermidis* is known to adhere preferably to polymers like PMMA.[36,37] This capability of coagulase-negative staphylococci to colonize and produce a biofilm on a biomaterial is probably an important virulence factor in causing foreign body infections, which infections are more indolent by nature.

The initial loss of two rabbits after cementing probably was caused by pulmonary embolism, due to the increased intramedullary pressure during injection of the cement.[16] Thorough lavage of the femoral canal and testing of the width of the reamed femur prevented this complication as much as possible.

It should be noted that, because the method used to culture and count bacteria has a detection limit of around 1000 CFU per gram of bone, very low numbers of bacteria could not be detected. All negative cultures were considered as '0' bacteria present in the sample, while theoretically a negative culture can be any culture below 1000 CFU. This might explain the relatively large standard errors at some inoculum doses in the control group. Such a minimal detection limit is a relative drawback of all studies that evaluate implant-related infections using a comparable plating and counting technique.[22,38-45] However, many studies do not mention the minimal detectable level of bacteria. Early experiments have shown that, in the presence of a foreign body, a number of 100 CFU of a staphylococcal strain is enough to produce an infection in man.[46] Waldvogel et al. (1991) stated that, because even clean wounds were found to be contaminated, the control of infection is more a quantitative than a qualitative problem.[47] Therefore, the value of antibiotic bone cement is to eliminate the bacteria to such low numbers that host defense mechanisms can clear the remaining infection. It is noteworthy that in present study the histological appearance of the rabbits with tobramycin-containing bone cement confirmed the negative bacteriological cultures. Especially, the recovery of variant and resistant strains such as small colony variants may be even more difficult due to their altered growth mode.[48] It has been reported that both *Staphylococcus aureus* and coagulase-negative staphylococci can develop resistance against gentamicin after use of gentamicin-containing cement or beads.[48,49] Adequate diagnosis of infection is a prerequisite to evaluate new biomaterials and operative techniques optimally, and, of course, to treat patients with an infected implant successfully.

In conclusion, this animal model shows that premixed tobramycin-containing bone cement can prevent implant bed infection in rabbits after contamination with *Staphylococcus aureus* Wood-46 and *Staphylococcus epidermidis* O-47.

References

1. Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Joint Surg [Am] 1996;78:512–523.
2. Sanzen L, Carlsson AS, Josefsson G, Lindberg LT. Revision operations on infected total hip arthroplasties. Two- to nine-year follow-up study. Clin Orthop 1988;229:165–172.
3. Ostendorf M, Malchau H. A report of 302 reoperations for deep infection in THR from the Swedish Hip Registration. 14th Euro Conf Biomater, The Hague; 1998.
4. Garvin KL, Fitzgerald RH Jr, Salvati EA, Brause BD, Nercessian OA, Wallrichs SL, Ilstrup DM. Reconstruction of the infected total hip and knee arthroplasty with gentamicin-impregnated Palacos bone cement. Instr Course Lect 1993;42: 293–302.
5. Fitzgerald RH Jr. Treatment of infected total hip replacement. Curr Opin Ortho 1994;5:26–30.
6. Fleer A, Timmerman CP, Besnier JM, Pascual A, Verhoef J. Surface proteins of coagulase-negative staphylococci: their role in adherence to biomaterials and in opsonization. J Biomater Appl 1990;5:154–165.
7. Naylor PT, Myrvik QN, Gristina A. Antibiotic resistance of biomaterial-adherent coagulase-negative and coagulase-positive staphylococci. Clin Orthop 1990;261:126–133.
8. Darouiche RO, Dhir A, Miller AJ, Landon GC, Raad II, Musher DM. Vancomycin penetration into biofilm covering infected prostheses and effect on bacteria. J Infect Dis 1994;170:720–723
9. Buchholz HW, Elson RA, Engelbracht E, Lodenkamper H, Rottger J, Siegel A. Management of deep infection of total hip replacement. J Bone Joint Surg [Br] 1981;63-B:342–353.
10. Whelton A. The aminoglycosides. Clin Orthop 1984;190:66–74.
11. Edson RS, Terrell CL. The aminoglycosides. Mayo Clin Proc 1991;66:1158–1164.
12. Lode H. Tobramycin: a review of therapeutic uses and dosing schedules. Curr Therapeut Res 1998;59:420–453.
13. Heck D, Rosenberg A, Schink Ascani M, Garbus S, Kiewitt T. Use of antibiotic-impregnated cement during hip and knee arthoplasty in the United States. J Arthroplasty 1995;10:470–475.
14. Davies JP, Harris WH. Effect of hand mixing tobramycin on the fatigue strength of Simplex P. J Biomed Mater Res 1991;25: 1409–1414.
15. Lautenschlager EP, Jacobs JJ, Marshall GW, Meyer PR Jr. Mechanical properties of bone cements containing large doses of antibiotic powders. J Biomed Mater Res 1976;10:929–938.
16. Nijhof MW, Dhert WJA, Tilman PBJ, Fleer A, Verbout AJ. Release of tobramycin from tobramycin-containing bone cement in bone and serum of rabbits. J Mater Sci Mater Med 1997;8:799–802.
17. Heilmann C, Gerke C, Perdreau–Remington F, Gotz F. Characterization of Tn917 insertion mutants of Staphylococcus epidermidis affected in biofilm formation. Infect Immun 1996;65:277–282.
18. Brown DFJ. Evaluation of the E test, a novel method of quantifying antimicrobial activity. J Antimicrob Chemother 1991;27:185–190.
19. Powles JW, Spencer RF, Lovering AM. Gentamicin release from old cement during revision hip arthroplasty. J Bone Joint Surg [Br] 1998;80:607–610.

20. Elson RA, Jephcott AE, McGechie DB, Verettas D. Bacterial infection and acrylic cement in the rat. J Bone Joint Surg [Br] 1977;59:452–457.
21. Petty W, Spanier S, Shuster JJ. Prevention of infection after total joint replacement. Experiments with a canine model. J Bone Joint Surg [Am] 1988;70:536–539.
22. Rodeheaver GT, Rukstalis D, Bono M, Bellamy W. A new model of bone infection used to evaluate the efficacy of antibiotic-impregnated polymethylmethacrylate cement. Clin Ortho 1983;178:303–311.
23. Schurman DJ, Trindade C, Hirshman HP, Moser K, Kajiyama G, Stevens P. Antibiotic-acrylic bone cement composites. Studies of gentamicin and Palacos. J Bone Joint Surg [Am] 1978;60:978–984.
24. Lynch M, Esser MP, Shelley P, Wroblewski BM. Deep infection in Charnley low-friction arthroplasty. Comparison of plain and gentamicin-loaded cement. J Bone Joint Surg [Br] 1987;69:355–360.
25. Josefsson G, Gudmundsson G, Kolmert L, Wijkstrom S. Prophylaxis with systemic antibiotics versus gentamicin bone cement in total hip arthroplasty. A five-year survey of 1688 hips. Clin Ortho 1990;253:173–178.
26. Josefsson G, Kolmert L. Prophylaxis with systemic antibiotics versus gentamicin bone cement in total hip arthroplasty. A ten-year survey of 1,688 hips. Clin Ortho 1993;292:210–214.
27. Espehaug B, Engesaeter LB, Vollset SE, Havelin LI, Langeland N. Antibiotic prophylaxis in total hip arthroplasty. Review of 10,905 primary cemented total hip replacements reported to the Norwegian arthroplasty register, 1987 to 1995. J Bone Joint Surg [Br] 1997;79:590–595.
28. Lacy MK, Nicolau DP, Nightingale CH, Quintiliani R. The pharmacodynamics of aminoglycosides. Clin Infect Dis 1998;27:23–27.
29. Eckman JB Jr, Henry SL, Mangino PD, Seligson D. Wound and serum levels of tobramycin with the prophylactic use of tobramycin-impregnated polymethylmethacrylate beads in compound fractures. Clin Ortho 1988;237:213–215.
30. Lyons VO, Henry SL, Faghiri M, Seligson D. Bacterial adherence to plain and tobramycin-laden polymethylmethacrylate beads. Clin Ortho 1992;278:260–264.
31. Brien WW, Salvati EA, Klein R, Brause B, Stern S. Antibiotic impregnated bone cement in total hip arthroplasty. An in vivo comparison of the elution properties of tobramycin and vancomycin. Clin Ortho 1993;296:242–248.
32. Pritchett JW, Bortel DT. Tobramycin-impregnated cement in total hip relacements. Orthop Rev. 1992;21:577–579.
33. Soto–Hall R, Saenz L, Tavernetti R, Cabaud HE, Cochran TP. Tobramycin in bone cement. An in-depth analysis of wound, serum, and urine concentrations in patients undergoing total hip revision arthroplasty. Clin Ortho 1983;175:60–64.
34. Hofmann AA, Kane KR, Tkach TK, Plaster RL, Camargo MP. Treatment of infected total knee arthroplasty using an articulating spacer. Clin Ortho 1995;321:45–54.
35. Cunningham R, Cockayne A, Humphreys H. Clinical and molecular aspects of the pathogenesis of Staphylococcus aureus bone and joint infections. J Med Microbio 1996;44:157–164.
36. Gristina AG, Naylor PT. Implant-associated infection. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, editors. Biomaterials science. An introduction to materials in medicine. San Diego: Academic; 1996. p 205–214.
37. Barth E, Myrvik WM, Wagner W, Gristina AG. In vitro and in vivo comparative colonization of Staphylococcus aureus and Staphylococcus epidermidis on orthopaedic implant materials. Biomater 1989;10;325–328.
38. Cremieux AC, Mghir AS, Bleton R, Manteau M, Belmatoug N, Massias L, Garry L, Sales N, Maziere B, Carbon C. Efficacy of sparfloxacin and autoradiographic diffusion pattern of [14C]Sparfloxacin in experimental Staphylococcus aureus joint prosthesis infection. Antimicrob Agents Chemother 1996;40: 2111–2116.
39. Cordero J, Munuera L, Folgueira MD. Influence of bacterial strains on bone infection. J Ortho Res 1996;14:663–667.
40. Curtis MJ, Brown PR, Dick JD, Jinnah RH. Contaminated fractures of the tibia: A comparison of treatment modalities in an animal model. J Ortho Res 1995;13:286–295.
41. Eerenberg JP, Patka P, Haarman HJ, Dwars BJ. A new model for posttraumatic osteomyelitis in rabbits. J Invest Surg 1994;7:453–465.
42. Fitzgerald RH Jr. Experimental osteomyelitis: description of a canine model and the role of depot administration of antibiotics in the prevention and treatment of sepsis. J Bone Joint Surg [Am] 1983;65:371–380.
43. Korkusuz F, Uchida A, Shinto Y, Araki N, Inoue K, Ono K. Experimental implant-related osteomyelitis treated by antibiotic-calcium hydroxyapatite ceramic composites. J Bone Joint Surg [Br] 1993;75:111–114.
44. Melcher GA, Claudi B, Schlegel U, Perren SM, Printzen G, Munzinger J. Influence of type of medullary nail on the development of local infection. An experimental study of solid and slotted nails in rabbits. J Bone Joint Surg [Br] 1994;76:955–959.
45. Isiklar ZU, Darouiche RO, Landon GC, Beck T. Efficacy of antibiotics alone for orthopaedic device related infections. Clin Orthop 1996;332:184–189.
46. Elek SD, Conen PE. The virulence of Staphylococcus pyogenes for man. Br J Exp Path 1957;38:573–586.
47. Waldvogel FA, Vaudaux PE, Pittet D, Lew PD. Perioperative antibiotic prophylaxis of wound and foreign body infections: microbial factors affecting efficacy. Rev Infect Dis 1991;13(Suppl 10):S782–789.
48. von Eiff C, Bettin D, Proctor RA, Rolauffs B, Lindner N, Winkelmann W, Peters G. Recovery of small colony variants of Staphylococcus aureus following gentamicin bead placement for osteomyelitis. Clin Infect Dis 1997;25:1250–1251.
49. Hope PG, Kristinsson KG, Norman P, Elson RA. Deep infection of cemented total hip arthroplasties caused by coagulase-negative staphylococci. J Bone Joint Surg [Br] 1989;71:851–855.