Journal of Antimicrobial Chemotherapy (2006) **57**, 301–305
doi:10.1093/jac/dki435
Advance Access publication 16 December 2005

JAC

Daptomycin treatment of *Staphylococcus aureus* experimental chronic osteomyelitis

Mark S. Rouse[1], Kerryl E. Piper[1], Melissa Jacobson[1], David J. Jacofsky[2], James M. Steckelberg[1] and Robin Patel[1,3]*

[1]Division of Infectious Diseases, Department of Internal Medicine, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, USA; [2]Department of Orthopaedic Surgery, Mayo Clinic College of Medicine, Rochester, MN 55905, USA; [3]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

Received 25 August 2005; returned 3 October 2005; revised 26 October 2005; accepted 6 November 2005

***Background*: Infection due to methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly common in nosocomial and community settings. Daptomycin is a cyclic lipopeptide anti-infective with activity against MRSA, approved for treatment of complicated skin and skin structure infections. Daptomycin may be useful in systemic or local treatment of chronic osteomyelitis.**

***Methods*: We measured mechanical strength of daptomycin- and vancomycin-loaded polymethylmethacrylate (PMMA), assayed *in vivo* release of daptomycin and vancomycin from daptomycin- and vancomycin-loaded PMMA, respectively, and compared the efficacy of two systemic doses of daptomycin with that of vancomycin, each with or without the respective anti-infective loaded into PMMA, using a rat model of MRSA chronic osteomyelitis.**

***Results*: Neither tensile nor compressive strength of PMMA was impacted by impregnation with these antimicrobials at a concentration of 7.5% by weight. The peak concentrations of daptomycin and vancomycin in rat tibial bone surrounding a 7.5% daptomycin- and vancomycin-loaded 3 mm PMMA bead were 178 and 49 mg/L, respectively. In the treatment of experimental osteomyelitis, rats assigned to no treatment, daptomycin 50 mg/kg subcutaneously twice daily, daptomycin 60 mg/kg subcutaneously twice daily, and vancomycin 50 mg/kg intraperitoneally twice daily had 6.4, 4.1, 4.0 and 4.5 median $\log_{10}$ cfu/g of bone at the end of 21 days of therapy. All systemic anti-infectives studied were more active than was no treatment. Daptomycin- or vancomycin-loaded PMMA did not, however, exhibit microbiological efficacy alone or adjunctively, as assessed 21 days after implantation.**

***Conclusions*: Daptomycin is released from PMMA *in vivo* at a rate similar to that of vancomycin. Systemic daptomycin is as active as vancomycin in a rat model of chronic MRSA experimental osteomyelitis.**

Keywords: polymethylmethacrylate, rats, vancomycin

Introduction

Osteomyelitis is challenging to treat. *Staphylococcus aureus* is the most common causative organism,[1] and methicillin resistance in *S. aureus* is increasingly common. Vancomycin is often used to treat bone and joint infection caused by methicillin-resistant *S. aureus* (MRSA). However, vancomycin-intermediate and vancomycin-resistant strains of *S. aureus* have been reported, and vancomycin administration may be inconvenient because of the need to monitor concentrations in serum, and may be associated with the occasional occurrence of adverse effects, such as marrow suppression, ototoxicity, nephrotoxicity, rash or linear IgA bullous dermatosis.[2]

Daptomycin, a recently FDA-approved antimicrobial agent with *in vitro* activity against *S. aureus*, including methicillin-resistant strains (MIC_{90} values typically ≤1 mg/L),[3] has a unique mechanism of action. It binds to bacterial cell membranes and effects rapid depolarization of membrane potential resulting in inhibition of protein, DNA and RNA synthesis. Daptomycin is a potentially attractive alternative to vancomycin for the treatment of

*Corresponding author. Tel: +1-507-255-6482; Fax: +1-507-284-9066; E-mail: patel.robin@mayo.edu

osteomyelitis because of once daily dosing, a lack of need for monitoring of concentrations in serum and its favourable toxicity profile.

Local delivery of antimicrobial agents, primarily via polymethylmethacrylate (PMMA), has become common practice in orthopaedic surgery over the past three decades.[4] Vancomycin along with the aminoglycosides gentamicin and tobramycin are the antimicrobial agents most commonly impregnated into PMMA.[4] Given that vancomycin resistance among staphylococci is an emerging problem, the possibility of mixing alternative antimicrobial agents into PMMA warrants exploration. We recently showed that daptomycin is released from PMMA *in vitro* at a rate similar to that of vancomycin.[5] This, together with the microbiological spectrum of daptomycin, suggests that daptomycin might be an alternative to vancomycin for impregnation of PMMA if daptomycin does not negatively impact the physical strength of PMMA, if daptomycin elutes from PMMA *in vivo* and if locally-delivered daptomycin demonstrates activity in the treatment of bone and joint infection.

We studied the mechanical strength of daptomycin-loaded PMMA, the *in vivo* release kinetics of PMMA-delivered daptomycin and the antimicrobial activity of daptomycin in the treatment of chronic MRSA osteomyelitis in an experimental rat model.

Materials and methods

Organism

A clinical MRSA isolate (IDRL no. 4293), with a daptomycin MIC of 0.5 mg/L and vancomycin MIC of 1.0 mg/L, was studied.

Antimicrobial agents

Daptomycin powder, supplied by Cubist Pharmaceuticals (Lexington, MA, USA), and vancomycin powder, supplied by Abbott Laboratories (Abbott Park, IL, USA), were studied.

PMMA loaded with daptomycin, vancomycin or no antimicrobial agent

PMMA was loaded with daptomycin or vancomycin by adding 750 mg of active daptomycin or vancomycin powder, respectively, to 10 g of the powder component of Simplex P® (Howmedica Osteonics, Limerick, Ireland) PMMA. After thorough mixing, 5 mL of liquid monomer was added, hand mixed for 2 min, set aside for 2 min and then formed into one of a variety of shapes, as described below. Unloaded (bland) PMMA was prepared as described but without antimicrobial agents.

Mechanical strength testing of PMMA loaded with daptomycin, vancomycin or no antimicrobial agent

Mechanical strength testing was performed according to FDA guidelines,[6] using a servohydraulic testing machine (Model 810; MTS Corporation, Minneapolis, MN, USA). Compressive strength to yield was determined according to ISO 5833 (E),[7] using antimicrobial agent-loaded or unloaded PMMA cylinders [6 mm (diameter) × 10 mm (length)] and a compression rate of 5 mm/min. The load at 2% offset from the Hookean portion of a continuous *x–y* plot of the load/displacement curve was used to calculate compressive strength. Tensile strength to yield was determined at a rate of 5 mm/min according to ASTM D638-03 using dumb-bell-shaped antimicrobial agent-loaded or bland PMMA (rectangular cross section area, 20.16 mm^2). An extensometer (Model 632; MTS Corporation) was used for strain measurement. The load at yield was used to calculate the tensile strength.

Daptomycin and vancomycin bioassays

Bioassays were performed in triplicate using *Micrococcus luteus* ATCC 9341 as the indicator organism. Concentrations of daptomycin or vancomycin were calculated by linear regression of the mean size of the zones of inhibition against the standard curve. The range of the standards was 4–32 mg/L in the daptomycin assay and 2–32 mg/L in the vancomycin assay. Standards for serum bioassay were prepared in pooled rat serum; standards for bone bioassay were prepared in homogenized rat tibia in water. Serum or bone specimens for testing were diluted in pooled rat serum or homogenized rat tibia/water suspension, respectively. The limits of detection for concentrations of antimicrobial agents in bone were 10 μg of daptomycin/g of bone and 5 μg of vancomycin/g of bone. The area under the concentration–time curve (AUC) was calculated from the mean concentrations by the trapezoidal rule.

In vivo release kinetics of daptomycin or vancomycin

Antimicrobial agent-loaded PMMA was pressed into a 3 mm bead mould and allowed to polymerize for 2 h. Beads weighing 22 mg with no visible surface imperfections were studied. Ten daptomycin- and 10 vancomycin-loaded beads were individually cultured in 25 mL of trypticase soy broth to assess sterility.

To confirm daptomycin and vancomycin activity following polymerization of PMMA, three daptomycin- and three vancomycin-loaded beads were individually crushed, homogenized using a T-25 homogenizer (IKA-Werke, Wilmington, NC, USA) in 2 mL of distilled water and incubated at 5°C for 48 h. The homogenate was assayed for daptomycin or vancomycin.

The use and handling of animals in these studies were reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee. *In vivo* release of daptomycin or vancomycin into bone surrounding daptomycin- or vancomycin-loaded beads was determined in 48 healthy male Wistar rats (175–200 g). A single daptomycin- or vancomycin-loaded bead was implanted in the left tibia in each rat. Three animals with a daptomycin-loaded and three with a vancomycin-loaded PMMA bead were sacrificed after each of 2, 4, 6, 8, 10, 14, 18 and 21 days following bead implantation. The left tibia was removed and a 1 cm length of bone centred on the bead was measured with a ruler and was sectioned. The bead was removed, the bone weighed, minced with a sterile ronguer, homogenized in 2 mL of water and incubated for 48 h at 5°C. The homogenate was assayed for daptomycin or vancomycin.

Experimental osteomyelitis

Experimental osteomyelitis was established in the proximal left tibia in 154 male Wistar rats (175–200 g), as previously described.[8] Animals were anaesthetized with ketamine and xylazine and the proximal third of the left tibia surgically exposed. A 0.5 mm hole was drilled into the medullary cavity. Fifty microlitres of morrhuate sodium, followed by 50 μL of saline containing 10^7 cfu of *S. aureus* were injected into the bone. The skin was closed with sutures and the wound sprayed with antiseptic plastifilm.

Twenty-eight days later, the animals underwent limited surgical debridement of the infection site by boring a 3 mm hole through cortical bone to the medullary cavity; antimicrobial therapy was then initiated. Two parenteral daptomycin regimens (50 or 60 mg/kg subcutaneously, twice daily) and a single vancomycin regimen (50 mg/kg intraperitoneally, twice daily) were administered for 21 days. Daptomycin doses were selected to simulate the AUC observed in humans after

administration of 6 or 8 mg/kg; vancomycin dose was selected to simulate time above the MIC observed in humans. In addition, some animals had a 3 mm PMMA bead loaded with daptomycin or vancomycin, or a bland PMMA bead, placed into the bone defect present after debridement.

Twelve hours after treatment, the animals were sacrificed and tibial bone within 5 mm of the bead was aseptically harvested and weighed. Any PMMA in the bone was gently removed. Bone specimens from one animal in each treatment group underwent haematoxylin and eosin staining to confirm the histological features of chronic osteomyelitis. Bone specimens from the remaining animals were processed for culture. The bone was homogenized, as described above; quantitative cultures were performed by preparing serial 10-fold dilutions and plating 0.1 mL of each dilution on sheep blood agar plates. Following incubation for 48 h in 5% CO_2 at 37°C, the colonies were counted and results expressed as mean $\log_{10}$ cfu/g of bone ± standard deviation. The entire remaining specimen was placed in 10 mL of trypticase soy broth and incubated for 48 h in 5% CO_2 at 37°C. Sterile cultures were assigned a value of 1 $\log_{10}$ cfu/g of bone.

MIC values of *S. aureus* recovered from bone were determined using standard methods;[9] daptomycin susceptibility testing was performed in medium supplemented with calcium chloride to contain 50 mg/L Ca^{2+}. Serum concentrations of vancomycin or daptomycin were determined following three doses of vancomycin or daptomycin, respectively, in infected rats. Serum was collected by tail vein phlebotomy from groups of five animals at various time intervals after administration of the third dose of daptomycin or vancomycin.

Statistics

Data were statistically compared using ANOVA and the Kruskal–Wallis test, using Bonferroni's correction for multiple comparisons.

Results

Mechanical strength testing of PMMA loaded with daptomycin, vancomycin or no antimicrobial agent

Compressive strength of bland ($n = 17$), daptomycin-loaded ($n = 18$) and vancomycin-loaded ($n = 18$) PMMA (mean ± standard deviation) was 113.4 ± 6.0, 106.9 ± 5.8 and 102.9 ± 4.6 MPa, respectively, exceeding the ISO 5833 (E)[7] specified minimum strength requirement of 70 MPa. Results of tensile strength testing for bland ($n = 8$), daptomycin-loaded ($n = 9$) and vancomycin-loaded ($n = 7$) PMMA revealed mean ± standard deviation values of 118.7 ± 9.2, 112.0 ± 8.8 and 114.6 ± 3.5 MPa, respectively. The tensile strength of daptomycin- or vancomycin-loaded PMMA was not significantly different from that of bland PMMA (ANOVA, $P = 0.23$).

In vivo release kinetics of daptomycin or vancomycin

All tested beads were sterile. The studies to confirm daptomycin and vancomycin activity following PMMA polymerization detected all the loaded amount of daptomycin and 96% of the loaded amount of vancomycin in the homogenate, indicating that the study antimicrobial agents remained biologically active following polymerization of PMMA. Mean concentrations of daptomycin and vancomycin in bone surrounding the antimicrobial agent-loaded PMMA beads are shown in Table 1.

Table 1. Mean antimicrobial concentration in bone within 5 mm of beads at various times after implantation of daptomycin- or vancomycin-loaded polymethylmethacrylate beads

	Mean antimicrobial concentration (μg/g of bone)	
Days after implantation	Daptomycin-loaded bead	Vancomycin-loaded bead
2	69 ± 13	27 ± 6
4	178 ± 24	49 ± 14
6	56 ± 19	32 ± 6
8	15 ± 3	<5
10	<10	<5
14	<10	<5
18	<10	<5
21	<10	<5

Experimental osteomyelitis

Five of 154 rats did not survive 28 days to begin antimicrobial therapy, and were not further analysed. Bone specimens from one animal in each treatment group were reviewed to confirm the histological features consistent with chronic osteomyelitis. An acute inflammatory response with microabscess formation was observed in all specimens. The results of treatment are plotted as mean $\log_{10}$ cfu/g of bone and standard deviation for each treatment group in Figure 1. Results of treatment with systemic daptomycin at either of the two doses studied or with vancomycin were significantly better than was no treatment ($P < 0.008$), but were not significantly different from each other ($P > 0.1$). There were no significant differences in results of treatment among the animals treated with daptomycin- or vancomycin-loaded beads or bland beads ($P = 0.52$). The combinations of systemic daptomycin with daptomycin-loaded PMMA or systemic vancomycin with vancomycin-loaded PMMA were significantly better than was no treatment ($P < 0.008$). No significant differences were detected between results of treatment with systemic daptomycin (either dose) and no bead, systemic vancomycin and no bead, systemic daptomycin (either dose) plus a daptomycin-loaded bead, or systemic vancomycin plus a vancomycin-loaded bead ($P = 0.73$).

The MIC values of the *S. aureus* recovered from animals after daptomycin and vancomycin treatment were 0.25–1.0 and 1.0–2.0 mg/L for daptomycin and vancomycin, respectively.

The mean concentrations in serum of daptomycin and vancomycin at various times after administration of the third dose of each treatment regimen are listed in Table 2. The AUC_{0-24} for 50 mg/kg daptomycin, 60 mg/kg daptomycin and vancomycin were 858, 982 and 88 μg·h/mL, respectively. The 24 h AUC/MIC ratios for 50 mg/kg daptomycin, 60 mg/kg daptomycin and vancomycin were 1716, 1964 and 88, respectively. The peak/MIC ratios for 50 mg/kg daptomycin, 60 mg/kg daptomycin and vancomycin were 404, 452 and 47 mg/L, respectively. The $\%t > \text{MIC}$ for vancomycin was 83%.

Discussion

Our study shows that treatment with parenteral daptomycin or vancomycin for 21 days results in significantly reduced numbers of bacteria in bone surrounding the infection site in a rat model of experimental osteomyelitis. Our results contrast with those of Luu *et al.*[10] who demonstrated that, in their rat model of chronic methicillin-susceptible *S. aureus* osteomyelitis, daptomycin

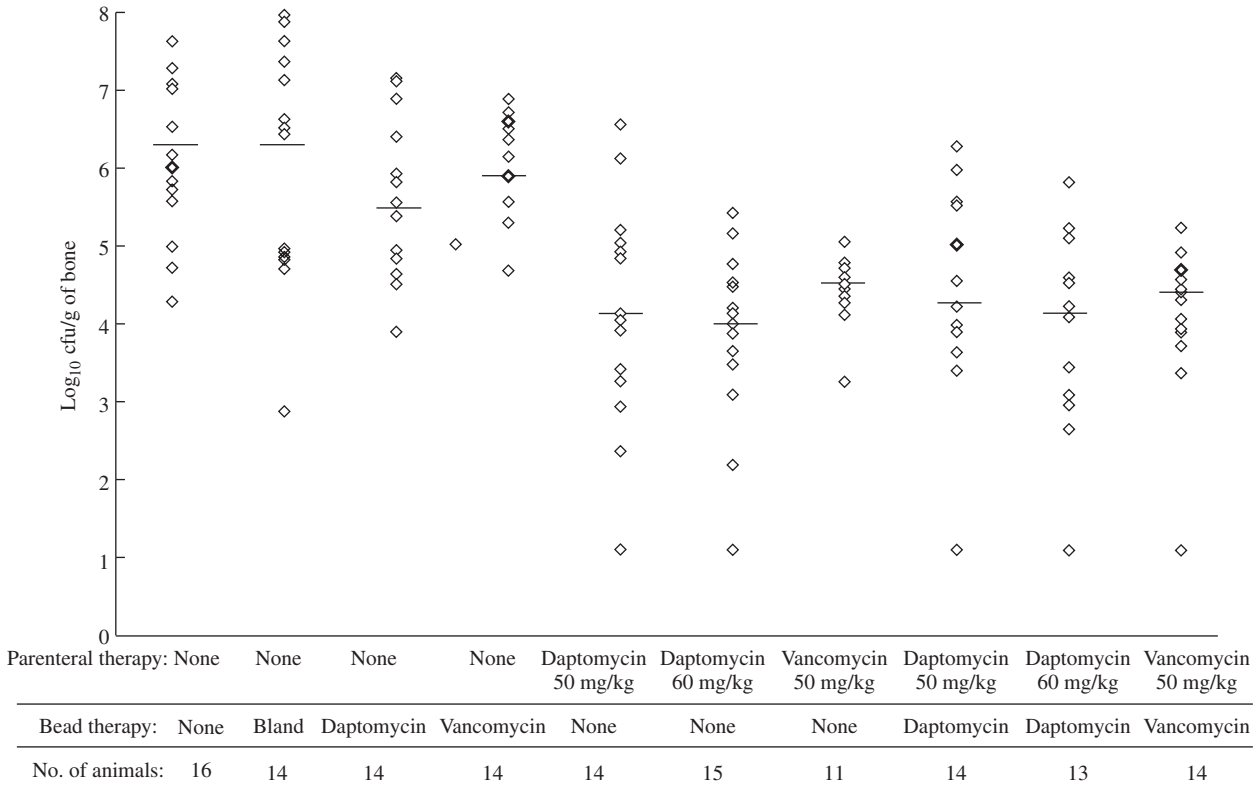

Figure 1. Results of treatment of MRSA experimental osteomyelitis. Horizontal bars indicate median values.

Table 2. Mean concentration in serum (in mg/L) at time after administration

		Time (h)					
Antimicrobial	Dose (mg/kg)	0.5	1	2	4	6	12
Daptomycin	50	–	169 ± 10.1	202 ± 17.2	87 ± 10.4	53 ± 9.3	all <4
Daptomycin	60	–	183 ± 22.3	226 ± 19.8	104 ± 28.0	68 ± 7.3	4.2 ± 1.3
Vancomycin	50	47 ± 8.2	26 ± 6.2	14 ± 4.4	8.2 ± 1.4	7.4 ± 2.4	all <2

10 mg/kg subcutaneously every 12 h resulted in similar numbers of bacteria in bone to those of untreated animals. In retrospect, their findings probably relate to underdosing. In a rat model of subcutaneously implanted tissue cages chronically infected with *S. aureus*, daptomycin 30 mg/kg subcutaneously every 24 h was as effective as vancomycin.[11] Safdar *et al*.[12] examined several doses of daptomycin in an experimental murine *S. aureus* thigh infection model and demonstrated concentration-dependent bactericidal activity. In their model, a mean peak/MIC ratio of 255 or a mean 24 h AUC/MIC ratio of 1061 was needed to effect a 2 $\log_{10}$ reduction *in vivo*. The calculated daptomycin peak/MIC and 24 h AUC/MIC ratios in our study were well-above these values; the daptomycin $AUC_{0–24}$ values in our study are comparable to those in humans receiving once daily daptomycin 6–8 mg/kg.[13] Similar to the results of our study, daptomycin has been shown to have comparable activity to vancomycin in a rabbit model of MRSA osteomyelitis.[14]

In our model, daptomycin and vancomycin MIC values of MRSA isolates recovered from animals following treatment with daptomycin and vancomycin, respectively, were within one doubling dilution of their pre-treatment MIC values, indicating a lack of detection of emergence of resistance.

We have previously shown that daptomycin is released from PMMA in a continuous flow chamber at a rate similar to that of vancomycin.[5,15] In the current study, we demonstrate similar release of daptomycin and vancomycin from PMMA *in vivo*. We also demonstrate that impregnation of PMMA with daptomycin (or vancomycin) does not affect tensile or compressive strength.

Despite the activity of systemic vancomycin and daptomycin in our model, neither effected a microbiological cure. It may be that more aggressive or repeat debridement, longer therapy or adjunctive locally delivered anti-infectives would have effected a cure. We postulated that locally delivered daptomycin or vancomycin alone (i.e. without systemic therapy) might have an effect in our model and that adjunctive locally delivered daptomycin or vancomycin (i.e. in addition to systemic therapy) might improve the activity of systemic therapy. We evaluated the use of locally delivered daptomycin and vancomycin to a limited extent. In our model, locally delivered daptomycin or vancomycin did not lead to a reduction in the number of bacteria in the bone surrounding the PMMA.

Although our data do not support the use of daptomycin or vancomycin impregnated beads in the management of *S. aureus* osteomyelitis, our study design had some limitations. The bone concentrations of vancomycin or daptomycin at the time of sacrifice were low. Whether findings would have been different had we sacrificed the infected animals after a shorter duration of treatment is unknown. If so, the clinical correlate would be that daptomycin- or vancomycin-loaded PMMA might need to periodically be replaced with a fresh specimen, or that a longer *in vivo* elution time (i.e. through alternative drug delivery systems)[16] should be pursued.

In summary, our study shows that adding 7.5% daptomycin or 7.5% vancomycin to PMMA does not affect tensile or compressive strength of PMMA, that daptomycin is released from PMMA *in vivo* at a rate similar to that of vancomycin and that systemic daptomycin is as active as vancomycin in the treatment of experimental MRSA osteomyelitis.

Acknowledgements

Presented in part at the Fifteenth European Conference on Clinical Microbiology and Infectious Diseases, Copenhagen, Denmark, 2005. Supported by Cubist Pharmaceuticals and the Forty-fifth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2005.

Transparency declarations

The authors drafted the manuscript which was reviewed by Cubist Pharmaceuticals prior to submission to the journal.

References

1. Lew DP, Waldvogel FA. Osteomyelitis. *N Engl J Med* 1997; **336**: 999–1007.

2. Solky BA, Pincus L, Horan RF. Vancomycin-induced linear IgA bullous dermatosis: morphology is a key to diagnosis. *Cutis* 2004; **73**: 65–7.

3. Schriever CA, Fernandez C, Rodvold KA *et al.* Daptomycin: a novel cyclic lipopeptide antimicrobial. *Am J Health Syst Pharm* 2005; **62**: 1145–58.

4. Hanssen AD, Spangehl MJ. Practical applications of antibiotic-loaded bone cement for treatment of infected joint replacements. *Clin Orthop* 2004; **1**: 79–85.

5. Hall EW, Rouse MS, Jacofsky DJ *et al.* Release of daptomycin from polymethylmethacrylate beads in a continuous flow chamber. *Diagn Microbiol Infect Dis* 2004; **50**: 261–5.

6. Center for Devices and Neurological Health. *Class II Special Controls Guidance Document: Polymethylmethacrylate (PMMA) Bone Cement; Guidance for Industry and FDA*. Rockville, MD: U.S. Dept of Health and Human Services, 2002: 1–16.

7. International Organization for Standardization. *Implants of Surgery—Acrylic Resin Cements.* Geneva: ISO, 2002: 1–15.

8. Patel R, Piper KE, Rouse MS *et al.* Linezolid therapy of *Staphylococcus aureus* experimental osteomyelitis. *Antimicrob Agents Chemother* 2000; **44**: 3438–40.

9. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Sixth Edition: Approved Standard M07-A6.* CLSI, Villanova, PA, USA, 2003.

10. Luu QN, Buxton TB, Nelson DR *et al.* Treatment of chronic experimental *Staphylococcus aureus* osteomyelitis with LY146032 and vancomycin. *Eur J Clin Microbiol Infect Dis* 1989; **8**: 562–3.

11. Vaudaux P, Francois P, Bisognano C *et al.* Comparative efficacy of daptomycin and vancomycin in the therapy of experimental foreign body infection due to *Staphylococcus aureus*. *J Antimicrob Chemother* 2003; **52**: 89–95.

12. Safdar N, Andes D, Craig WA. *In vivo* pharmacodynamic activity of daptomycin. *Antimicrob Agents Chemother* 2004; **48**: 63–8.

13. Dvorchik BH, Brazier D, DeBruin MF *et al.* Daptomycin pharmacokinetics and safety following administration of escalating doses once daily to healthy subjects. *Antimicrob Agents Chemother* 2003; **47**: 1318–23.

14. Mader JT, Adams K. Comparative evaluation of daptomycin (LY146032) and vancomycin in the treatment of experimental methicillin-resistant *Staphylococcus aureus* osteomyelitis in rabbits. *Antimicrob Agents Chemother* 1989; **33**: 689–92.

15. Perry AC, Rouse MS, Khaliq Y *et al.* Antimicrobial release kinetics from polymethylmethacrylate in a novel continuous flow chamber. *Clin Orthop Relat Res* 2002; **403**: 49–53.

16. Liu SJ, Wen-Neng Ueng S, Lin SS *et al.* *In vivo* release of vancomycin from biodegradable beads. *J Biomed Mater Res* 2002; **63**: 807–13.