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Efficacies of Ceftobiprole Medocaril and Comparators in a Rabbit Model of Osteomyelitis Due to Methicillin-Resistant *Staphylococcus aureus*▽

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The pharmacokinetics and distribution into bone tissue of ceftobiprole in uninfected New Zealand White rabbits were determined after subcutaneous administration of the prodrug ceftobiprole medocaril. Serum exposure (maximum concentration of the drug in serum, trough concentration, area under the concentration-time curve) to ceftobiprole at 20 and 80 mg/kg was dose proportional, and there was no accumulation of ceftobiprole following repeated (every 6 h [q6h]) injections of the antibiotic. Ceftobiprole titers in the tibial matrix and marrow were 3.2 ± 1.3 μg/g and 11.2 ± 6.5 μg/g, respectively, in uninfected animals treated with 20 mg/kg of the antibiotic and 13.4 ± 7.3 μg/g and 66.3 ± 43.2 μg/g, respectively, in uninfected animals treated with 80 mg/kg of the antibiotic. No differences in ceftobiprole titers were observed between right and left tibiae for either bone matrix or marrow. The efficacies of 4 weeks of treatment with ceftobiprole (40 mg/kg administered subcutaneously [s.c.] q6h), vancomycin (30 mg/kg administered s.c. q12h), or linezolid (60 mg/kg administered orally q8h) were compared, using a rabbit model of methicillin-resistant *Staphylococcus aureus* tibial osteomyelitis. After treatment with ceftobiprole, the bacterial titers in all infected left tibiae from evaluable rabbits were below the level of detection, whereas only 73% of infected left tibiae from vancomycin- or linezolid-treated animals had bacterial titers below the level of detection; the mean titers of ceftobiprole were 3 to 5 times higher in infected left tibiae than in uninfected right tibiae. These results indicate that ceftobiprole provided effective parenteral treatment of osteomyelitis in this rabbit model.

Osteomyelitis is a serious infectious disease that, absent appropriate therapy, can lead to chronic pain, septic arthritis, disabling joint destruction, abnormal bone remodeling, vascular compromise, amyloidosis, and Marjolin's ulcers. Inflammatory reactions to the invading pathogen form an exudate that collects locally and compresses blood vessels, leading to osteonecrosis. Infections localized to the sequestrum are remarkably refractory to antibiotics, which are unable to penetrate these floating islands of bone.

Staphylococcus aureus is the predominant pathogen in osteomyelitic infections (12); in patients with staphylococcal bacteremia, metastatic bone involvement occurs in greater than 10% of cases (5). While community-acquired osteomyelitis is caused primarily by methicillin-susceptible *S. aureus*, the incidence of osteomyelitis due to community-acquired methicillin-resistant *S. aureus* (MRSA) is on the rise (3), with MRSA as a frequent cause of nosocomial bone infections (22). Once acute osteomyelitis becomes chronic, the infection may continue for decades in a stable state, with intermittent exacerbations or relapses after prolonged periods of complete inactivity (12).

The administration of parenteral antibiotics to patients with acute osteomyelitis is a useful adjunct to surgical debridement of the infected bone and surrounding tissue. The prolonged antibiotic therapy (≥4 weeks) prescribed for treatment of osteomyelitis (12, 25) derives both from difficulties associated with antibiotic delivery (tissue revascularization requires up to 3 weeks following bone debridement) and from the biofilm nature of osteomyelitic infections (in which bacteria tend to be highly resistant to both antibiotics and host immunovigilance) (6). The need for antibiotic therapy over such protracted periods raises important issues relating to drug toxicity and emergence of antibiotic resistance during treatment. There is a clear and present need for new antibiotics which are not only effective for short-term therapy but which are also safe for long-term therapy of indications such as osteomyelitis. Toward this end, we have examined the efficacy of ceftobiprole, a novel broad-spectrum pyrrolidinone-3-ylidenemethyl cephem with potent anti-MRSA activity (4), together with two other anti-MRSA agents, vancomycin and linezolid, in a rabbit model of MRSA tibial osteomyelitis.

MATERIALS AND METHODS

Reagents. Normal New Zealand White (NZW) rabbit serum was purchased from Gibco BRL (Gaithersburg, Md.), and oxacillin was obtained from the Sigma Chemical Co. (St. Louis, MO). The vancomycin hydrochloride (Novaplus) used was a product of Novation LLC (Irving, TX), and the linezolid (Zyvox for oral suspension) was a product of the Pharmacia & Upjohn Co. (Kalamazoo, MI). Ceftobiprole and its water-soluble prodrug ceftobiprole medocaril were obtained from Basilea Pharmaceutica AG (Basel, Switzerland). Tetradeuterated ceftobiprole and tetradeuterated ceftobiprole medocaril were synthesized at Basilea Pharmaceutica China Ltd. (Haimen, People's Republic of China). The sterile water used for injection USP was from Abbott Laboratories (Chicago, IL), and the sterile water used for irrigation and sterile physiological saline were from the Baxter Healthcare Corp. (Deerfield, IL). All other chemicals utilized in this study were of reagent or analytical grade. Solutions were prepared with distilled or deionized water and sterilized prior to use.

Susceptibility testing. *S. aureus* strain 168-1 is a clinical isolate recovered from a patient treated for osteomyelitis (32, 33). MICs were determined by broth macrodilution (17) in cation-adjusted Mueller-Hinton broth (CAMHB; Difco

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Laboratories, Detroit, MI), and minimal bactericidal concentrations were determined in CAMHB according to CLSI (formerly NCCLS) guidelines (16). All other microbiological manipulations were performed using aseptic techniques in accordance with accepted practices.

Quantification of drug concentrations. Vancomycin and linezolid concentrations in sera from the animals dosed with these antibiotics (20-μl serum samples diluted with normal rabbit serum) were quantified by disc diffusion (10) on plates of soft nutrient agar seeded with 10^6 CFU/ml of *Bacillus cereus* ATCC 11778 (30°C, 13.5 h).

Ceftobiprole and ceftobiprole medocaril concentrations in biological samples were quantified by reverse-phase high-pressure liquid chromatography (RP-HPLC) coupled online with tandem mass spectrometry (MS/MS) as described previously (2), using tetradeuterated ceftobiprole and tetradeuterated ceftobiprole medocaril as internal standards. The limits of quantification were 0.05 μg/ml for plasma, 0.23 μg/g for bone matrix, and 0.60 μg/g for marrow. During method validation, overall reproducibility varied less than 13%, with a day-to-day variability of less than 15%.

Exploratory dose range pharmacokinetics of ceftobiprole. All procedures involving animals were approved by the Animal Care and Use Committee of the University of Missouri—Columbia. Two groups of uninfected NZW rabbits (Ray Nicholl's Rabbitry, Lumberton, TX), five animals per group, received subcutaneous (s.c.) boluses of ceftobiprole medocaril dissolved in 20 mM citrate buffer (pH 5.0) every 6 h (q6h) for 4 days (16 injections per animal per group). One group of rabbits received doses of ceftobiprole medocaril corresponding to 20 mg/kg of ceftobiprole, whereas the other group received doses of ceftobiprole medocaril corresponding to 80 mg/kg of ceftobiprole. Blood samples were taken from the lateral or medial ear vein at 1 h, 3 h, and 6 h (trough concentration) after the initial bolus and 1 h after the last injection. Immediately after they were collected, the samples were transferred into sterile, prechilled 1.5-ml microcentrifuge tubes containing 10 μl of 2 M citric acid and 25 μl of 0.5 M disodium EDTA dihydrate per milliliter of blood. The tubes were mixed by gentle inversion and centrifuged (1,500 × *g*, 4°C, 15 min), and the plasma was stored at −80°C pending analysis by RP-HPLC/MS/MS. Values for peak plasma concentrations (C_{max}) and AUC (areas under the curve) were obtained using WinNonlin version 4.0.1 (Pharsight Corporation, Mountain View, CA).

Following collection of the final samples, the rabbits were anesthetized by the s.c. injection of 45 mg/kg of ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and 5.5 mg/kg of xylazine (X-Ject SA, Burns Veterinary Supply, Inc., Rockville Center, NY) and euthanized by intravenous injection (0.1 ml/lb) of Beuthanasia-D Special (Schering-Plough Animal Health Corp., Kenilworth, NJ). Both tibiae were excised from each animal; after the removal of contiguous soft tissue, the tibiae were thoroughly rinsed with a gentle stream of sterile water and stored in sterile 50-ml plastic centrifuge tubes at −80°C. After all the tibiae had been harvested, the bones were fragmented, using sterile single-action rongeurs (jaw size, 5 mm). Marrow from each tibia was placed in a sterile plastic tube, while the remaining matrices were broken into small chips (chip surface area, ~0.5 mm^2) and transferred to sterile plastic tubes (31). The bone matrices and marrow were stored at −80°C pending workup.

Tibial marrow (ca. 200-mg samples) was diluted with 0.1 M ammonium formate (pH 5.0) and sonicated for 10 s. Tibial matrix fragments (ca. 100-mg samples) were suspended in 0.5 M perchloric acid ($HClO_4$), sonicated for 20 s, and then shaken at 4°C for 7 h. Aliquots (100 μl) of matrix and marrow extracts were diluted with ammonium formate buffer containing tetradeuterated ceftobiprole, tetradeuterated ceftobiprole medocaril, and 0.5 M $HClO_4$ and centrifuged, and 100 μl of each supernatant was analyzed by RP-HPLC/MS/MS.

Induction of tibial osteomyelitis and antibiotic treatment. An animal model of long-bone infection intermediate between hematogenous and chronic contiguous-focus osteomyelitis, producing a progressive osteomyelitis accompanied by periosteal reaction, lytic bone lesions, sequestra, and involucra (14), was employed in these studies.

S. aureus strain 168-1 was incubated overnight at 37°C in CAMHB supplemented with 40 μg/ml of oxacillin, and the cells were then centrifuged (1,000 × *g*, 4°C, 10 min) and resuspended in sterile phosphate-buffered saline (pH 7.2 to 7.4) to yield 10^7 cells/ml in phosphate-buffered saline, determined by using a Petroff-Hauser counting chamber.

Seventy NZW rabbits, ranging in age from 8 to 12 weeks and in weight from 2.0 to 3.5 kg, were anesthesized, and an 18-gauge needle was inserted percutaneously through the lateral aspect of the left tibial metaphysis into the intramedullary cavity; 0.15 ml of 5% sodium morrhuate (American Reagent Laboratories, Inc., Shirley, NY), 0.1 ml of *S. aureus* suspension, and 0.2 ml of sterile physiological saline were then injected sequentially. The infection was allowed to progress for 2 weeks, spreading proximally and distally to involve the entire tibia, at which time bilateral tibial radiographs were taken to gauge the severity of the osteomyelitis and scored according to a visual scale (14) by three treatment-blinded investigators whose ratings for each group at each time point were averaged. Then the rabbits were divided into several groups. According to the study rules established by the Animal Care and Use Committee, each rabbit could receive only one X-ray exposure per examination period, so if an image was unclear or indecisive the tibial radiograph for that rabbit for that time point was excluded from consideration.

Group 1 (sample size, $n = 15$) was left untreated for the duration of the study; group 2 ($n = 15$) was treated s.c. q12h for 4 weeks with 30 mg/kg vancomycin, group 3 ($n = 15$) was treated p.o. q8h for 4 weeks with 60 mg/kg linezolid, and group 4 ($n = 15$) was treated s.c. q6h for 4 weeks with ceftobiprole medocaril corresponding to 40 mg/kg of ceftobiprole. Peak and trough levels of vancomycin were obtained for six rabbits sampled at 0.5 h and 12 h, respectively, after administration of the first dose, whereas peak and trough levels of linezolid were obtained for six rabbits at 0.5 h and 8 h, respectively, after administration of the first dose.

Following the treatment period, rabbits in groups 2 to 4 received no further antibiotic for 2 weeks. Bilateral tibial radiographs of animals in groups 1 to 4 were obtained and scored at the end of antibiotic therapy (6 weeks after infection) and at sacrifice (8 weeks after infection). C_{max} and C_{trough} values for each antibiotic for the animals in groups 2 to 4 were determined after the initial antibiotic administration.

The rabbits in group 5 ($n = 10$) were treated s.c. q6h for 1 week only with ceftobiprole medocaril corresponding to 40 mg/kg of ceftobiprole (identical to that used for group 4). Three hours after the last dose, a 1-ml blood sample was taken from each rabbit in the group, the animals were then euthanized, and the left and right tibiae were harvested. The concentrations of ceftobiprole in plasma, bone matrix, and marrow were determined.

Rabbits are particularly prone to gastrointestinal disturbances consequent to long-term antibiotic therapy, so all animals were monitored weekly for changes in weight. Animals displaying symptoms of gastrointestinal distress received nutritional supplements plus a probiotic preparation.

Quantification of bacteria in tibial matrix and marrow. Marrow and the intramedullary canal of bilateral tibiae were swabbed with sterile cotton-tipped applicators. The inoculated applicators were streaked onto plates of trypticase soy agar II supplemented with 5% (vol/vol) defibrinated sheep blood (BBL; Becton, Dickinson and Co., Sparks, MD) and then placed into tubes containing 5 ml of trypticase soy broth (BBL). The plates and tubes were incubated at 37°C for 24 h, and the presence or absence of growth in both media was recorded.

Marrow from each tibia from each rabbit was deposited into sterile 50-ml centrifuge tubes and weighed. Matrix from each tibia from each rabbit was cut into 0.5 cm^2 chips, placed in sterile 50-ml centrifuge tubes, and weighed. Physiological saline was added at a ratio of 3 ml of saline per gram of bone matrix or marrow. Bone matrix and marrow suspensions were vortexed for 2 min and serially diluted with sterile physiological saline. Aliquots (20-μl) were plated onto blood agar and incubated at 37°C, and colonies were counted after 24 h. The limit of detection for viable counts was 50 CFU/ml, corresponding to 150 CFU/g of bone matrix and 200 CFU/g of marrow (31).

Statistical analysis of data. Means ± standard deviations were calculated using Microsoft Office Excel 2003 SP2. A two-tailed Student's *t* test was used to determine whether there were significant differences in the bacterial counts in the matrix and marrow from the left and right tibiae in rabbits from different groups and to compare radiographic scores among the first, second, and third sets of radiographs. Fisher's exact test was used to assess the significance of bacterial clearance among pairwise treatment groups.

RESULTS

Antimicrobial susceptibility. The MIC/minimal bactericidal concentrations of ceftobiprole, vancomycin, and linezolid for *S. aureus* strain 168-1 were 0.39/6.25 μg/ml, 0.78/6.25 μg/ml, and 1.56/12.5 μg/ml, respectively.

Exploratory dose range pharmacokinetics of ceftobiprole. Due to the rapid cleavage of ceftobiprole medocaril to ceftobiprole in vivo, the prodrug was undetectable in any plasma or bone samples, whereas ceftobiprole was quantifiable in plasma and bone from rabbits treated with ceftobiprole medocaril. Serum exposure to ceftobiprole was nearly dose proportional for the 20 mg/kg ($n = 5$; C_{max}, 23.8 ± 4.2 μg/ml; C_{trough}, 2.7 ± 0.5 μg/ml; AUC, 65.3 ± 7.1 μg · h/ml) and 80 mg/kg ($n = 5$; C_{max}, 83.3 ± 9.9 μg/ml; C_{trough}, 7.8 ± 3.4 μg/ml; AUC, 215.0 ± 37.1 μg · h/ml)

TABLE 1. Radiographic scores for MRSA-infected rabbits in groups 1 to 4

Time of infection or postinfection	Radiographic score for indicated group:[a]			
	1 (no treatment)	2 (vancomycin)	3 (linezolid)	4 (ceftobiprole)
Time of infection	0 (15)	0 (15)	0 (15)	0 (15)
2 wk postinfection (start of treatment)	2.6 ± 0.8 (14)	2.5 ± 0.6 (15)	3.1 ± 0.2 (11)	3.0 ± 0.6 (13)
6 wk postinfection (end of treatment)	3.1 ± 0.8 (13)	3.2 ± 0.7 (15)	2.3 ± 0.8 (11)	2.8 ± 0.8 (13)
8 wk postinfection (2 wk posttreatment)	2.6 ± 1.1 (14)	2.4 ± 1.2 (15)[b]	1.6 ± 1.1 (7)[c]	2.4 ± 0.8 (13)[d]

[a] Values are given as means ± standard deviations. Numbers in parentheses are sample sizes.
[b] $P > 0.7$ compared to score at start of treatment.
[c] $P < 0.005$ compared to score at start of treatment.
[d] $P < 0.05$ compared to score at start of treatment.

doses. There was no accumulation of ceftobiprole during repeated q6h injections of the antibiotic into uninfected rabbits, as indicated by the C_{max} values for ceftobiprole 1 h after the first injection (see above) and the sixteenth injection (for a 20-mg/kg dose, $n = 5$, $C_{max} = 22.1 \pm 4.3$ μg/ml; for an 80-mg/kg dose, $n = 4$, $C_{max} = 58.9 \pm 14.9$ μg/ml).

One hour after the sixteenth q6h s.c. treatment, the titers of ceftobiprole in the tibial matrix and marrow of uninfected rabbits were 3.2 ± 1.3 μg/g ($n = 5$) and 11.2 ± 6.5 μg/g ($n = 5$), respectively, in animals treated with 20 mg/kg of antibiotic and 13.4 ± 7.3 μg/g ($n = 5$) and 66.3 ± 43.2 μg/g ($n = 4$), respectively, in animals treated with 80 mg/kg of the antibiotic. No differences in ceftobiprole content were observed between the right and left tibiae.

On the basis of this exploratory study in uninfected rabbits, a ceftobiprole dosing regimen of 40 mg/kg of ceftobiprole administered s.c. q6h was selected for an efficacy study in osteomyelitic rabbits. Using 40-mg/kg doses of ceftobiprole, a C_{max} of ~40 μg/ml and an AUC of ~110 μg · h/ml were predicted for rabbits, with ceftobiprole concentrations in both plasma and tibiae expected to exceed the MIC for at least 40% of the dosing interval. The ceftobiprole dosage used, 40 mg/kg, is a clinically relevant one, following from C_{max} and AUC values of 34.2 μg/ml and 116 μg · h/ml, respectively, observed in healthy volunteers following infusion of a therapeutic dose of 500 mg of ceftobiprole over a period of 1 h (24).

Tibial osteomyelitis model. The peak and trough values for ceftobiprole in the plasma of infected rabbits (group 4) after initial dosing were 76.8 ± 17.3 μg/ml ($n = 4$) and 5.7 ± 4.8 μg/ml ($n = 5$), respectively. The peak, trough, and AUC values for vancomycin (group 2; $n = 6$) were 6.4 ± 0.9 μg/ml, 3.4 ± 0.6 μg/ml, and 58 μg · h/ml, respectively, whereas those for linezolid (group 3; $n = 6$) were 27.2 ± 1.3 μg/ml, 17.0 ± 1.4 μg/ml, and 173 μg · h/ml, respectively.

Three hours after the final dosing with ceftobiprole (corresponding to 50% of the dosing interval), the plasma concentration of this antibiotic in rabbits of group 5 ($n = 8$) was 4.9 ± 1.5 μg/ml. In infected left tibiae ($n = 9$), the matrix and marrow concentrations of ceftobiprole were 0.9 ± 1.1 μg/g and 12.8 ± 17.7 μg/g, respectively, whereas in uninfected right tibiae ($n = 9$) ceftobiprole concentrations in matrix and marrow were 0.3 ± 0.5 μg/g and 2.4 ± 4.2 μg/g, respectively.

Radiographic results reflect the rate and extent of bone reconstruction and remodeling, which in osteomyelitis always lag behind bacterial clearance. Initial radiographic scores for linezolid-treated rabbits (group 3) and for ceftobiprole-treated rabbits (group 4) were significantly higher than for the untreated controls (group 1) or for the vancomycin-treated animals (group 2) (Table 1). By 8 weeks after the start of treatment (2 weeks posttreatment), rabbits in the control group showed zero radiographic improvement, compared to a 4% mean radiographic improvement for vancomycin treatment (not significant), a 48% mean radiographic improvement for linezolid treatment, and a 20% mean radiographic improvement for ceftobiprole treatment; however, at this time point, there were no significant differences in mean radiographic scores among the four treatment groups.

The bacterial titers in the right tibiae of all infected rabbits were below the level of detection, whereas in untreated infected animals (group 1), nearly all (13 of 14) left tibiae were positive for MRSA 8 weeks postinfection. The bacterial concentration in the infected tibial matrix of the untreated rabbits was $1.04 \pm 2.63 \times 10^7$ CFU/g, whereas the bacterial concentration in the infected tibial marrow of this group was $0.57 \pm 1.12 \times 10^9$ CFU/g. In contrast, 4 of 15 left tibiae (27%) from the vancomycin-treated animals proved positive for MRSA (matrix, $0.84 \pm 3.12 \times 10^7$ CFU/g; marrow, $0.64 \pm 2.41 \times 10^9$ CFU/g), and 3 of 11 left tibiae (27%) from linezolid-treated animals proved positive for MRSA (matrix, $0.18 \pm 5.64 \times 10^7$ CFU/g; marrow, $2.02 \pm 6.39 \times 10^9$ CFU/g). However, no MRSA (0%) were recovered from any of the 13 left tibiae from the evaluable rabbits treated with ceftobiprole.

Nearly all the rabbits treated with ceftobiprole or linezolid exhibited symptoms of gastrointestinal distress (decreased appetite, dehydration, diarrhea, and/or weight loss) beginning 1 to 1.5 weeks after the commencement of antibiotic treatment. To reverse this condition, the animals in the ceftobiprole and linezolid arms received 8 g of a probiotic preparation, Probios (Vets Plus, Inc., Knapp, WI), administered p.o. q24h in conjunction with a nutritional supplement (Ensure; Ross Products Division, Abbott Laboratories, Columbus, OH). The weight changes for the animals in groups 1 to 4 during the 8 weeks of the study are presented in Table 2. The untreated control animals (group 1) showed the greatest mean weight gain (0.43 kg), whereas the mean weight gain by the vancomycin-treated rabbits (0.26 kg) matched that of the ceftobiprole-treated rabbits.

The data for 7 (11.7%) of the 60 infected rabbits comprising groups 1 to 4 were excluded from the final analysis due to death or other reasons. One rabbit in the control group (group 1) was excluded due to the inadvertent administration of vancomycin. Two rabbits in the ceftobiprole arm (group 4) were excluded, one due to death from a handling accident on the first day of treatment and the other due to death from apparent

TABLE 2. Weight changes for MRSA-infected rabbits in groups 1 to 4

Time of infection or postinfection	Weight for indicated group:[a]			
	1 (no treatment)	2 (vancomycin)	3 (linezolid)	4 (ceftobiprole)
Time of infection	2.81 ± 0.43 (14)	2.84 ± 0.30 (15)	2.43 ± 0.15 (11)	2.84 ± 0.29 (13)
2 wk postinfection (start of treatment)	2.79 ± 0.47 (14)	2.77 ± 0.43 (15)	2.35 ± 0.21 (11)	2.64 ± 0.31 (13)
4 wk postinfection (midtreatment)	2.96 ± 0.45 (14)	2.84 ± 0.47 (15)	2.44 ± 0.20 (11)	2.73 ± 0.23 (13)
6 wk postinfection (end of treatment)	3.16 ± 0.43 (14)	2.93 ± 0.50 (15)	2.36 ± 0.25 (11)	2.86 ± 0.23 (13)
8 wk postinfection (2 wk posttreatment)	3.24 ± 0.40 (14)	3.10 ± 0.45 (15)	2.42 ± 0.27 (11)	3.07 ± 0.25 (13)

[a] Weights are given in kilograms and are means ± standard deviations. Numbers in parentheses are sample sizes.

gastroenterocolitis on day 7. In the linezolid arm (group 3), five rabbits died from apparent gastroenterocolitis; however, since one of these five rabbits succumbed only 4 days before the end of the study, the data collected for that rabbit were retained, while data for the four linezolid-treated rabbits that died earlier were excluded from the final analysis.

DISCUSSION

The number of treatment days for osteomyelitis is greater than for any other bacterial infection (26); nonetheless, treatment of osteomyelitis relying predominantly on long-term antibiotic therapy has been disappointing, with recurrence rates of about 30% (26). Sterilization of bone matrix and marrow by antibiotics alone is extremely difficult to achieve; animal models of osteomyelitis, which correlate reasonably well with human disease in terms of chronicity and radiographic and histological changes, have demonstrated repeatedly that viable pathogens are recoverable from infected bones following prolonged treatment with antibiotics (13, 14, 18, 19–21, 23, 31–33). In the present study, ceftobiprole, vancomycin, and linezolid each proved significantly more efficacious in clearing MRSA infections than no treatment (Table 3). However, ceftobiprole stands apart from other antibiotics, including recently approved drugs such as linezolid, daptomycin, and tigecycline, insofar as monotherapy with ceftobiprole at a clinically relevant concentration resulted, within the limits of detectability, in a 100% microbiologic cure in the MRSA-infected rabbits available for evaluation. Pairwise comparisons of the microbiologic cure levels for the rabbits treated with ceftobiprole and vancomycin ($P = 0.07$) or with ceftobiprole and linezolid ($P = 0.08$), while not quite reaching the 0.05 level of significance, are nonetheless very encouraging for the efficacy of treatment of MRSA osteomyelitis with ceftobiprole (Table 3).

In the ceftobiprole-treated rabbits, the percentage of time the plasma drug concentration exceeds the MIC, the pharmacokinetic descriptor correlating most closely with efficacy (1), exceeded 40% in both plasma and infected tibiae (24), whereas in the linezolid-treated animals, the 24-h AUC/MIC, the pharmacokinetic driver for efficacy (7), was 214.

TABLE 3. Pairwise comparisons of clearance of MRSA from infected tibiae after different treatments

Treatment	% MRSA clearance (t_1:t_2)[a]	Sample size (n_1:n_2)	*P* value
Untreated:vancomycin	7:73	14:15	<0.01
Untreated:linezolid	7:73	14:11	<0.01
Untreated:ceftobiprole	7:100	14:13	<0.01
Vancomycin:linezolid	73:73	15:11	0.34
Vancomycin:ceftobiprole	73:100	15:13	0.07
Linezolid:ceftobiprole	73:100	11:13	0.08

[a] Determined by Fisher's exact test. t, treatment.

The dosing regimens for ceftobiprole and linezolid employed in this study were clinically relevant. On the basis of a pilot pharmacokinetic study, ceftobiprole administered s.c. at a dose of 40 mg/kg was projected to produce a C_{max} of ~40 μg/ml and an AUC of ~110 μg · h/ml, which compares favorably with the C_{max} of 34.2 μg/ml and AUC of 116 μg · h/ml observed in healthy volunteers when a therapeutic dose of 500 mg of ceftobiprole (as ceftobiprole medocaril) was infused over 1 h (24). For linezolid administered p.o. at a dose of 60 mg/kg, the estimated 24-h AUC value of 334 μg · h/ml for rabbits was similar to that observed for humans when the oxazolidinone was administered p.o. or intravenously at a therapeutic dose of 600 mg (Zyvox prescribing information; Pharmacia & Upjohn Co., March 2007).

In this study, the 24-h AUC/MIC of vancomycin for the rabbits of group 2 was 148. The dosing regimen for vancomycin employed in the present study (30 mg/kg s.c. q12h for 4 weeks) is comparable to dosing regimens used in other studies of experimental rabbit *S. aureus* osteomyelitis (13, 20, 31). Quantification of vancomycin serum concentrations in treated animals verified that the antibiotic titers exceeded the MIC for the infecting pathogen throughout the treatment period.

Lew and Waldvogel (12) reported that β-lactam antibiotics penetrate bone at 10 to 20% of serum levels. In experiments with uninfected rabbits, steady-state ceftobiprole concentrations were 13 to 16% of the 1-h serum C_{max} in bone matrix, whereas steady-state ceftobiprole concentrations after 4 days of dosing were 47 to 80% of the 1-h serum C_{max} in marrow. Ceftobiprole concentrations were higher in infected bones than in uninfected bones; similar results for vancomycin have been reported (30). The reason for this difference with ceftobiprole is not clear; however, for vancomycin, Wilson and Mader (30) speculate that it cannot be attributed to enhanced vascularization of infected bone, since the blood flow in osteomyelitic bones is reduced compared to that in uninfected bone.

Linezolid has approximately 100% oral bioavailability, and the mean peak concentrations in bone are reportedly 60% of plasma concentrations (9). However, linezolid is bacteriostatic toward staphylococci, whereas vancomycin and ceftobiprole are bactericidal (4), and bactericidal drugs are preferred for osteomyelitic infections (28). In addition, the usefulness of linezolid for long-term treatment is compromised by serious side effects, most notably myelosuppression and optic and peripheral neuropathies, possibly related to the inhibition of mi-

tochondrial protein synthesis (11). Vancomycin is often the drug of choice for treatment of MRSA infections, but it has slow killing effects, with strain-dependent bactericidal activity (4), and long-term treatment with this glycopeptide incurs a risk of serious renal impairment (8, 29). Moreover, the emergence of vancomycin-resistant enterococci and vancomycin-intermediate *S. aureus* strains consequent to overuse of vancomycin and the recent appearance of vancomycin-resistant clinical isolates of *S. aureus* highlight the need for prudent use of glycopeptides for long-term treatment of gram-positive pathogens. β-lactam antibiotics, on the other hand, are generally considered safe, with an extensive history of use and a low incidence of reported serious adverse effects. The refractoriness of staphylococci to selection for endogenous resistance to ceftobiprole (4, 27) suggests that emergence of resistance during a course of therapy with this cephalosporin would be a rare event. The pharmacokinetic and safety profiles of ceftobiprole medocaril (15), plus its broad antibacterial spectrum, including MRSA, suggest that ceftobiprole could become a candidate for parenteral treatment of osteomyelitis.

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REFERENCES

1. **Andes, D., and W. A. Craig.** 2000. *In-vivo* pharmacodynamics of RO 63–9141 against multiple bacterial pathogens. Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1079.
2. **Azoulay-Dupuis, E., J. P. Bédos, J. Mohler, A. Schmitt-Hoffmann, M. Schleimer, and S. Shapiro.** 2004. Efficacy of BAL5788, a prodrug of cephalosporin BAL9141, in a mouse model of acute pneumococcal pneumonia. Antimicrob. Agents Chemother. **48:**1105–1111.
3. **Bocchini, C. E., K. G. Hulten, E. O. Mason, Jr., B. E. Gonzalez, W. A. Hammerman, and S. L. Kaplan.** 2006. Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. Pediatrics **117:**433–440.
4. **Bogdanovich, T., L. M. Ednie, S. Shapiro, and P. C. Appelbaum.** 2005. Antistaphylococcal activity of ceftobiprole, a new broad-spectrum cephalosporin. Antimicrob. Agents Chemother. **49:**4210–4219.
5. **Bouza, E., and P. Muñoz.** 1999. Micro-organisms responsible for osteoarticular infections. Ballière's Best Pract. Res. Clin. Rheumatol. **13:**21–35.
6. **Brady, R. A., J. G. Leid, A. K. Camper, J. W. Costerton, and M. E. Shirtliff.** 2006. Identification of *Staphylococcus aureus* proteins recognized by the antibody-mediated immune response to a biofilm infection. Infect. Immun. **74:**3415–3426.
7. **Craig, W. A.** 2003. Basic pharmacodynamics of antibacterials with clinical applications to the use of β-lactams, glycopeptides, and linezolid. Infect. Dis. Clin. N. Am. **17:**479–501.
8. **Ginzburg, E., N. Namias, M. Brown, S. Ball, S. M. Hameed, and S. M. Cohn.** 2000. Gram positive infection in trauma patients: new strategies to decrease emerging Gram-positive resistance and vancomycin toxicity. Int. J. Antimicrob. Agents **16**(Suppl. 1)**:**S39–S42.
9. **Harwood, P. J., and P. V. Giannoudis.** 2004. The safety and efficacy of linezolid in orthopaedic practice for the treatment of infection due to antibiotic-resistant organisms. Expert Opin. Drug Safety **3:**405–414.
10. **Jabés, D., G. Candiani, G. Romanó, C. Brunati, S. Riva, and M. Cavaleri.** 2004. Efficacy of dalbavancin against methicillin-resistant *Staphylococcus aureus* in the rat granuloma pouch infection model. Antimicrob. Agents Chemother. **48:**1118–1123.
11. **Leach, K. L., S. M. Swaney, J. R. Colca, W. G. McDonald, J. R. Blinn, L. M. Thomasco, R. C. Gadwood, D. Shinabarger, L. Xiong, and A. S. Mankin.** 2007. The site of action of oxazolidinone antibiotics in living bacteria and in human mitochondria. Mol. Cell **26:**393–402.
12. **Lew, D. P. and F. A. Waldvogel.** 2004. Osteomyelitis. Lancet **364:**369–379.
13. **Mader, J. T., and K. Adams.** 1989. Comparative evaluation of daptomycin (LY146032) and vancomycin in the treatment of experimental methicillin-resistant *Staphylococcus aureus* osteomyelitis in rabbits. Antimicrob. Agents Chemother. **33:**689–692.
14. **Mader, J. T., and K. J. Wilson.** 1983. Comparative evaluation of cefamandole and cephalothin in the treatment of experimental *Staphylococcus aureus* osteomyelitis in rabbits. J. Bone Joint Surg. **65:**507–513.
15. **Murthy, B., and A. Schmitt-Hoffmann.** 2008. Pharmacokinetics and pharmacodynamics of ceftobiprole, an anti-MRSA cephalosporin with broad-spectrum activity. Clin. Pharmacokinet. **47:**21–33.
16. **NCCLS.** 1999. Methods for determining bactericidal activity of antimicrobial agents. Approved guideline M26-A. NCCLS, Wayne, Pa.
17. **NCCLS.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6, 6th edition. NCCLS, Wayne, Pa.
18. **Norden, C. W., and E. A. Gill.** 1990. Cefepime for treatment of experimental chronic osteomyelitis due to *Staphylococcus aureus*. J. Infect. Dis. **162:**1218–1219.
19. **Norden, C. W., K. Niederriter, and E. M. Shinners.** 1986. Treatment of experimental chronic osteomyelitis due to *Staphylococcus aureus* with teicoplanin. Infection **14:**136–138.
20. **Norden, C. W., and M. Shaffer.** 1983. Treatment of experimental chronic osteomyelitis due to *Staphylococcus aureus* with vancomycin and rifampin. J. Infect. Dis. **147:**352–357.
21. **Patel, R., K. E. Piper, M. S. Rouse, and J. M. Steckelberg.** 2000. Linezolid therapy of *Staphylococcus aureus* experimental osteomyelitis. Antimicrob. Agents Chemother. **44:**3438–3440.
22. **Rayner, C. R., L. M. Baddour, M. C. Birmingham, C. Norden, A. K. Meagher, and J. J. Schentag.** 2004. Linezolid in the treatment of osteomyelitis: results of compassionate use experience. Infection **32:**8–14.
23. **Rouse, M. S., K. E. Piper, M. Jacobson, D. J. Jacofsky, J. M. Steckelberg, and R. Patel.** 2006. Daptomycin treatment of *Staphylococcus aureus* experimental chronic osteomyelitis. J. Antimicrob. Chemother. **57:**301–305.
24. **Schmitt-Hoffmann, A., B. Roos, J. Spickermann, M. Roehrli, and R. Pypstra.** 2005. Influence of dose and infusion duration on the time above MIC of BAL9141 in healthy volunteers. Clin. Microbiol. Infect. **11**(Suppl. 2)**:**273.
25. **Swiontkowski, M. F., D. P. Hanel, N. B. Vedder, and J. P. Schwappach.** 1999. A comparison of short- and long-term intravenous antibiotic therapy in the postoperative management of adult osteomyelitis. J. Bone Joint Surg. Br. **81:**1046–1050.
26. **Tice, A. D., P. A. Hoaglund, and D. A. Shoultz.** 2003. Risk factors and treatment outcomes in osteomyelitis. J. Antimicrob. Chemother. **51:**1261–1268.
27. **Vaudaux, P., A. Gjinovci, M. Bento, D. Li, J. Schrenzel, and D. P. Lew.** 2005. Intensive therapy with ceftobiprole medocaril of experimental foreign-body infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **49:**3789–3793.
28. **Washington, J. A.** 1981. Bactericidal tests, p. 715–728. *In* J. A. Washington (ed.), Laboratory procedures in clinical microbiology. Springer-Verlag, New York, NY.
29. **Wicklow, B. A., M. R. Ogborn, I. W. Gibson, and T. D. Blydt-Hansen.** 2006. Biopsy-proven acute tubular necrosis in a child attributed to vancomycin intoxication. Pediatr. Nephrol. **21:**1194–1196.
30. **Wilson, K. J., and J. T. Mader.** 1984. Concentration of vancomycin in bone and serum of normal rabbits and those with osteomyelitis. Antimicrob. Agents Chemother. **25:**140–141.
31. **Yin, L.-Y., L. Lazzarini, F. Li, C. M. Stevens, and J. H. Calhoun.** 2005. Comparative evaluation of tigecycline and vancomycin, with and without rifampicin, in the treatment of methicillin-resistant *Staphylococcus aureus* experimental osteomyelitis in a rabbit model. J. Antimicrob. Chemother. **55:**995–1002.
32. **Yin, L. Y., J. H. Calhoun, T. C. Li, J. Alder, and S. X. Zhang.** 2006. Comparative efficacies of vancomycin and Cubicin, a novel lipopeptide antibiotic, in the treatment of methicillin-resistant *Staphylococcus aureus* osteomyelitis: studies with a rabbit model. Abstr. 44th Infect. Dis. Soc. Am. Annu. Meet., abstr. 358.
33. **Yin, L. Y. J. Calhoun, T. Thomas, and E. Wirtz.** 2007. Comparative efficacies of telavancin, vancomycin and linezolid in a rabbit model of methicillin-resistant *Staphylococcus aureus* osteomyelitis. Abstr. 17th ECCMID/25th ICC, Munich, Germany, poster 1769.