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 an uninfected New Zealand White rabbit 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 titer 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 titer 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 leads to osteoporosis, amyloidosis, and Marjolin’s ulcers. Inflammatory reactions to the invading pathogen form an exudate that leads to osteoporosis, amyloidosis, and Marjolin’s ulcers. Inflammatory reactions to the invading pathogen form an exudate that leads to osteoporosis, amyloidosis, and Marjolin’s ulcers. Inflammatory reactions to the invading pathogen form an exudate that leads to osteoporosis, amyloidosis, and Marjolin’s ulcers.

**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...
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 cefito-

prole medocaril as internal standards. The limits of quantification were 0.05 μg/ml for plasma, 0.23 μg/g for bone matrix, and 0.06 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 subcutane-
ous (s.c.) boluses 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 microcen-
trifuge 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 inver-

tion 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 plasma concen-
trations (C_max) and AUC (areas under the curve) were obtained using WinNon-
lin version 4.0.1 (Porvoo, Finland). 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 mm² chips, placed in sterile 50-ml centrifuge tubes, and weighed. Phys-

iological 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 inoculated 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).

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

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

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 $C_{\text{max}}$ of $\sim40 \mu g/ml$ and an AUC of $\sim110 \mu g \cdot h/ml$ were predicted for rabbits, with ceftobiprole concentrations in both plasma and tibias 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 $C_{\text{max}}$ and AUC values of $34.2 \mu g/ml$ and $116 \mu g \cdot h/ml$, 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 $76.8 \pm 17.3 \mu g/ml$ ($n = 4$) and $5.7 \pm 4.8 \mu g/ml$ ($n = 5$), respectively. The peak, trough, and AUC values for vancomycin (group 2; $n = 6$) were $6.4 \pm 0.9 \mu g/ml$, $3.4 \pm 0.6 \mu g/ml$, and $58 \mu g \cdot h/ml$, respectively, whereas those for linezolid (group 3; $n = 6$) were $27.2 \pm 1.3 \mu g/ml$, $17.0 \pm 1.4 \mu g/ml$, and $173 \mu g \cdot h/ml$, 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 $4.9 \pm 1.5 \mu g/ml$. In infected left tibias ($n = 9$), the matrix and marrow concentrations of ceftobiprole were $0.9 \pm 1.1 \mu g/g$ and $12.8 \pm 17.7 \mu g/g$, respectively, whereas in uninfected right tibias ($n = 9$), ceftobiprole concentrations in matrix and marrow were $0.3 \pm 0.5 \mu g/g$ and $2.4 \pm 4.2 \mu g/g$, 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 tibias of all infected rabbits were below the level of detection, whereas in untreated infected animals (group 1), nearly all (13 of 14) left tibias were positive for MRSA 8 weeks postinfection. The bacterial concentration in the infected tibial matrix of the untreated rabbits was $1.04 \pm 2.63 \times 10^6$ CFU/g, whereas the bacterial concentration in the infected tibial marrow of this group was $0.57 \pm 1.12 \times 10^6$ CFU/g. In contrast, 4 of 15 left tibias (27%) from the vancomycin-treated animals proved positive for MRSA (matrix, $0.84 \pm 3.12 \times 10^7$ CFU/g; marrow, $0.64 \pm 2.41 \times 10^7$ 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, $2.02 \pm 6.39 \times 10^7$ 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, Probius (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 (17.7%) 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.

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

<table>
<thead>
<tr>
<th>Time of infection or postinfection</th>
<th>1 (no treatment)</th>
<th>2 (vancomycin)</th>
<th>3 (linezolid)</th>
<th>4 (ceftobiprole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 wk postinfection (start of treatment)</td>
<td>$2.6 \pm 0.8 (14)$</td>
<td>$2.5 \pm 0.6 (15)$</td>
<td>$3.1 \pm 0.2 (11)$</td>
<td>$3.0 \pm 0.6 (13)$</td>
</tr>
<tr>
<td>6 wk postinfection (end of treatment)</td>
<td>$3.1 \pm 0.8 (13)$</td>
<td>$3.2 \pm 0.7 (15)$</td>
<td>$2.3 \pm 0.8 (11)$</td>
<td>$2.8 \pm 0.8 (13)$</td>
</tr>
<tr>
<td>8 wk postinfection (2 wk posttreatment)</td>
<td>$2.6 \pm 1.1 (14)$</td>
<td>$2.4 \pm 1.2 (15)^b$</td>
<td>$1.6 \pm 1.1 (7)^c$</td>
<td>$2.4 \pm 0.8 (13)^d$</td>
</tr>
</tbody>
</table>

*Values are given as means ± standard deviations. Numbers in parentheses are sample sizes.

$b P > 0.05$ 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.
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 historical 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.

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$_{\text{max}}$ of ~40 µg/ml and an AUC of ~110 µg · h/ml, which compares favorably with the C$_{\text{max}}$ 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$_{\text{max}}$ in bone matrix, whereas steady-state ceftobiprole concentrations after 4 days of dosing were 47 to 80% of the 1-h serum C$_{\text{max}}$ 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-
to mitochondrial 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 mediocaril (15), plus its broad antibacterial spectrum, including a course of therapy with this cephalosporin would be a rare incidence of reported serious adverse effects. The refractoriness 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 refractoryness of staphylococci to selection for endogenous resistance to cefotibiprole (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 cefotibiprole medocaril (15), plus its broad antibacterial spectrum, including MRSA, suggest that cefotibiprole could become a candidate for parenteral treatment of osteomyelitis.

ACKNOWLEDGMENT

This study was supported by a grant from Baselica Pharmaceutica AG, Basel, Switzerland.

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