Intensive Therapy with Ceftobiprole Medocaril of Experimental Foreign-Body Infection by Methicillin-Resistant *Staphylococcus aureus*

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The therapeutic activity of ceftobiprole medocaril, the water-soluble prodrug of ceftobiprole, was compared to that of vancomycin in a rat tissue cage model of chronic methicillin-resistant *Staphylococcus aureus* (MRSA) foreign-body infection. The MICs and MBCs of ceftobiprole and vancomycin in Mueller-Hinton broth for strain MRGR3 were 1 and 4 and 1 and 2 \(\mu\)g/ml, respectively. In vitro elimination rates of strain MRGR3 of 4 and 8 \(\mu\)g/ml of ceftobiprole or vancomycin were equivalent. After 2 weeks of infection, mean \(\pm\) standard error of the mean viable counts of strain MRGR3 were 6.83 \(\pm\) 0.11 log CFU/ml of tissue cage fluid \((n = 87)\). High-dose regimens of ceftobiprole medocaril (equivalent to 150 mg/kg of ceftobiprole) or 50 mg/kg vancomycin produced nearly identical average peak and trough levels of ceftobiprole and vancomycin in tissue cage fluid, which exceeded the MBC of either antibiotic towards strain MRGR3 for \(\geq 75\%\) of each dosing interval. After 7 days of therapy with ceftobiprole medocaril or vancomycin, average counts of MRGR3 decreased significantly \((P < 0.02)\) by 0.68 \(\pm\) 0.28 \((n = 29)\) and 0.88 \(\pm\) 0.22 \((n = 28)\) log CFU/ml of tissue cage fluid, respectively, compared with cages of untreated animals, but were not significantly different from each other. No resistant mutants were detected on ceftobiprole-supplemented agar following therapy with this cephalosporin. The in vivo activity of ceftobiprole medocaril against chronic MRSA foreign-body infections was equivalent to that of vancomycin and did not lead to the emergence of resistant subpopulations.

Infections due to *Staphylococcus aureus* associated with foreign implants, such as orthopedic prostheses and permanently inserted catheters, are very difficult to manage by antimicrobial chemotherapy. Semisynthetic penicillins, imipenem, vancomycin, fluoroquinolones, and glycopeptides show limited or no significant efficacy in the clinic or experimental therapy of prosthetic infections, and treatment with these drugs for this indication often result in clinical failures \((4, 45)\). The increasing prevalence of clinical isolates of methicillin-resistant *S. aureus* (MRSA), which frequently display multidrug resistance against all semisynthetic penicillins, penems, macrolides, aminoglycosides, and fluoroquinolones, is a worldwide problem \((8, 16, 31)\). Multidrug-resistant *S. aureus* is of particular concern because of its widespread occurrence, intrinsic virulence, and high adaptability to diverse environments \((8, 31)\). This problem has been compounded recently by the emergence of clinical isolates exhibiting intermediate \((19–21, 24, 28, 29, 31, 39, 40, 46)\) or high \((5, 11, 47)\) levels of resistance to glycopeptides, engendering the risk of virtually untreatable infections. Global dispersal of multidrug-resistant staphylococci mandates continual development of novel bactericidal agents able to combat such dangerous pathogens.

Recent development of \(\beta\)-lactams targeting penicillin-bind- ing protein 2’ \((\text{PBP}2’)\), which is chiefly responsible for methicillin resistance in *S. aureus*, represents an important advance in the field of antimicrobial chemotherapy. Ceftobiprole (formerly known as BAL9141) is a pyrrolidinone cephalosporin with high affinities for penicillin-binding proteins including PBP2’. Additional assets of ceftobiprole are its refractoriness to hydrolysis by class A and class C \(\beta\)-lactamasestes \((22)\), and recalcitrance towards development of endogenous resistance \((9, 18, 23, 27)\). Ceftobiprole exhibits excellent in vitro activity towards a wide array of gram-negative \((22, 25, 26)\) and gram-positive \((15, 18, 22, 26)\) pathogens, including MRSA and its vancomycin-intermediate or vancomycin-resistant derivatives \((5, 9, 14, 15, 18, 22, 26)\). Both animal \((3, 9, 18, 22)\) and human studies \((37, 38)\) point towards ceftobiprole as a promising candidate for treatment of MRSA infections.

We previously showed the usefulness of a rat tissue cage model of *S. aureus* chronic foreign-body infections for evaluating various categories of antimicrobial agents such as vancomycin \((32)\), teicoplanin \((36)\), imipenem \((35)\), daptomycin \((43)\), and several fluoroquinolones including floroxacin \((32)\), sparflaxcin, teflaxacin, and ciprofloxacin \((7)\) and levofloxacin and trovafloxacin \((44)\).

The present study aimed at evaluating the therapeutic potential of ceftobiprole and its propensity for emergence of resistance compared to the reference anti-MRSA agent vancomycin, in the rat tissue cage model of MRSA chronic foreign-body infection.

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MATERIALS AND METHODS

Bacterial strains. MRSA strain MRGR3, isolated in 1978 from an intensive care patient with catheter-related sepsis and selected for its unique virulence properties in the rat model of chronic tissue cage infections (7, 12, 32, 35, 36, 44), was used for in vitro and animal studies. This strain is resistant to multiple antibiotics, including methicillin, gentamicin, tetracycline, and chloramphenicol, but is susceptible to fluoroquinolones and rifampin (32). Methicillin-susceptible S. aureus (MSSA) quality control strain ATCC 29253 was also used for some in vitro experiments (22).

Antimicrobial agents. Ceftobiprole ([weight purity, 90%] and its water-soluble prodrug ceftobiprole medocaril (formerly known as BAL5788; weight purity 89.2%) were supplied by Basilea Pharmaceutica AG (Basel, Switzerland). Ceftobiprole medocaril, targeted to release 150 mg ceftobiprole/kg body weight, for high-dose regimens the prodrug solution contained 90 mg/ml of the ceftobiprole (high-dose) of the prodrug in 10 ml of citrate buffer, pH 5.0. Solubilization of the prodrug medocaril, targeted to release 150 mg ceftobiprole/kg body weight, were prepared within 30 min of use by dissolving either 300 mg (low-dose) or 900 mg (high-dose) of the prodrug in 10 ml of citrate buffer, pH 5.0. Vigorous vortexing the ceftobiprole solution was diluted with 1,782 ml of AR grade water.

For in vivo studies, fresh stock solutions of prodrug ceftobiprole medocaril were prepared within 30 min of use by dissolving either 300 mg (low-dose) or 900 mg (high-dose) of the prodrug in 10 ml of citrate buffer, pH 5.0. Solubilization of ceftobiprole medocaril was facilitated by mild sonication for a few minutes in a water bath. For low-dose regimens the prodrug solution contained 30 mg/ml of ceftobiprole medocaril, targeted to release 50 mg ceftobiprole/kg body weight, for high-dose regimens the prodrug solution contained 90 mg/ml of the ceftobiprole medocaril, targeted to release 150 mg ceftobiprole/kg body weight.

Vancomycin for intravenous infusion (Lilly, Giessen, Germany) was dissolved, stored at 4°C, and used within 48 h, as recommended by the manufacturer.

MICs and MBCs. The MICs and MBCs of ceftobiprole for MRSA strain MRGR3 or MSSA strain 25923 were determined by broth macrodilution according to National Committee for Clinical Laboratory Standards guidelines (33). Concentrations of ceftobiprole in cation-adjusted Mueller-Hinton broth (CAMHB, Difco, Detroit, Mich.) ranged from 8 down to 0.125 μg/ml and the bacterial inoculum (ca. 10³ CFU/ml) was obtained from 100-fold dilutions of 3-h log-phase cultures of either strain MRGR3 or control strain ATCC 29253 grown in CAMHB.

To minimize possible carryover effects of ceftobiprole during MBC determinations, 100-μl portions from all tubes with no visible growth were subcultured, either undiluted or serially diluted 10-fold in saline, on Mueller-Hinton agar (MHA) for 36 to 48 h at 37°C. The MBC was defined as the lowest concentration that killed 99.9% of the original inoculum.

Killing kinetic studies. Sterile plastic tubes containing 1 ml of CAMHB supplemented with either 4 or 8 μg/ml of ceftobiprole were incubated at 37°C with ca. 10⁶ CFU of MRSA strain MRGR3 or MSSA strain 25923 (obtained from 3-h log-phase cultures) either in static conditions or (when indicated) a shaking water bath. The number of viable organisms was determined by subculturing 100 μl of undiluted or serially diluted portions of broth on MHA after 0, 2, 4, 6, and 24 h of incubation. Colonies were counted using an automated colony counter (Counter- trium flash, IUL Instruments) after 48 h of incubation at 37°C. The detection limit was 2 log₁₀ CFU/ml.

To evaluate the impact of tissue cage fluid proteins on the bactericidal activities of either 4 or 8 μg/ml of ceftobiprole, the elimination rate of strain MRGR3 was also recorded in tubes containing 1 ml of a mixture of CAMHB and sterile tissue cage fluid (pooled from 10 different cages of unaffected animals) in a 3:1 ratio.

Single-step resistance mutation frequency. A freshly prepared stock solution of ceftobiprole (1 mg/ml) was admixed with melting MHA at 45°C. Aliquots (100 μl) of serial dilutions of washed overnight cultures of MRSA strain MRGR3 (10⁶ to 10¹ CFU) were plated in duplicate onto antibiotic-free MHA and MHA containing 4 μg/ml or 8 μg/ml ceftobiprole. After 48 h at 37°C plates were inspected for staphyllococcal colonies.

Pharmacokinetic properties of ceftobiprole in rat tissue cage fluids. A preliminary pharmacokinetic evaluation was performed to determine a dosing regimen for prodrug ceftobiprole medocaril yielding ceftobiprole levels in tissue cage fluids of at least 4 μg/ml (corresponding to the MBC of ceftobiprole for MRSA strain MRGR3) for at least 60% of the dosing interval. A low-dose regimen, delivering 50 mg-eq. of ceftobiprole per kg of body weight, and a high-dose regimen, delivering 150 mg-eq. of ceftobiprole per kg of body weight, were administered intraperitoneally every 12 hours over 1 week to six uninfected rats. Ceftobiprole levels in tissue cage fluids for both regimens were determined at 0, 2, 4, 6, and 12 h on day 4 (for a mid-therapy assessment of tissue cage fluid levels) and on day 7 (to assess possible accumulation of ceftobiprole in tissue cage fluids).

RESULTS

In vitro studies. The MICs and MBCs of ceftobiprole and vancomycin for MRSA strain MRGR3 in CAMHB were 1 and 4 and 2 and 1 μg/ml, respectively. The MIC and MBC of ceftobiprole for MSSA strain 25923 were 0.25 and 1 μg/ml, respectively.

Time-kill studies showed equivalent reductions in the viable counts of strain MRGR3 by 4 and 8 μg/ml of either cefotibeprole or vancomycin at all time points, averaging 1.5 and ≥3 log₁₀ CFU/ml at 6 and 24 h, respectively (data not shown). Additional controls comparing MRSA strain MRGR3 with MSSA strain 25923 (22) showed similar elimination rates of 4 and 8 μg/ml of ceftobiprole, and no difference was observed for cultures incubated in static or shaken conditions (data not shown). Reductions in viable counts of strain MRGR3 by 4 and 8 μg/ml of ceftobiprole in CAMHB supplemented with 50% tissue cage fluid were similar to those obtained in CAMHB alone (data not shown), indicating that the in vitro elimination rate of strain MRGR3 by ceftobiprole is unaffected by tissue cage fluid components.

Plating 10⁶ to 10⁸ CFU of MRSA strain MRGR3 onto MHA containing 4 or 8 μg/ml ceftobiprole completely suppressed bacterial growth during 48 h. Under these conditions, therefore, the single-step mutation frequency of strain MRGR3 for
ceftobiprole is $<10^{-8}$, even at a concentration as low as the MBC (4 μg/ml) for the test strain.

**Pharmacokinetics of ceftobiprole in rat tissue cages.** To determine the adequate dosing regimen of ceftobiprole medocaril yielding local ceftobiprole concentrations ≥4 μg/ml (which is the MBC of ceftobiprole for MRSA strain MRGR3) in tissue cage fluids for at least 60% of the dosing interval (for twice a day, 7 h), two different regimens of the prodrug (designed to deliver 50 or 150 mg-eq/kg of ceftobiprole) were compared, referred to as low-dose and high-dose regimens of ceftobiprole medocaril, respectively.

The mean peak and trough levels of ceftobiprole in tissue cage fluids ($n = 6$) of animals treated every 12 hours with low-dose ceftobiprole/kg were 4.11 and 0.79 μg/ml, respectively, on day 4 and 5.81 and 0.47 μg/ml, respectively, on day 7 (Fig. 1). These results indicated that the low-dose regimen was inadequate for therapy, since tissue cage fluid levels of ceftobiprole exceeded the MBC (4 μg/ml) for $<2$ h ($<17\%$) of the dosing interval. In contrast, mean peak and trough levels of ceftobiprole in tissue cage fluids ($n = 6$) of animals treated every 12 hours with 150 mg-eq/kg of ceftobiprole/kg were 16.14 and 2.40 μg/ml, respectively, on day 4 and 22.60 and 1.67 μg/ml, respectively, on day 7 (Fig. 1). Analysis of the clearance data for ceftobiprole in tissue cage fluids for the high-dose regimen indicated bactericidal levels (≥4 μg/ml) towards MRSA strain MRGR3 for at least 9 h (75%) of each dosing interval. Thus, the high-dose regimen of ceftobiprole medocaril was chosen for treatment studies of tissue cages infected with MRSA strain MRGR3.

Average peak and through levels of vancomycin in tissue cage fluids have been determined previously (32) as 12 and 2 μg/ml at 4 and 12 h, respectively, after intraperitoneal administration every 12 hours of 50 mg/kg of this glycopeptide.

**Treatment of chronic tissue cage infections.** At the onset of therapy, average bacterial counts for cages infected with strain MRGR3 were 6.88 ± 0.18 log$_{10}$ CFU/ml for controls ($n = 30$), 6.56 ± 0.18 log$_{10}$ CFU/ml for animals receiving ceftobiprole medocaril ($n = 29$), and 7.07 ± 0.18 log$_{10}$ CFU/ml for animals receiving vancomycin ($n = 28$). These three sets of bacterial counts did not differ significantly from one another ($P = 0.15$).

At the end of the 7-day treatment period, bacterial counts in tissue cages of control animals showed a slight albeit nonsignificant increase of 0.18 ± 0.20 log$_{10}$ CFU/ml ($n = 30$). Compared to the control group, antibiotic treatment led to significant ($P < 0.02$) reductions in bacterial counts in tissue cage fluids of 0.68 ± 0.28 log$_{10}$ CFU/ml ($n = 29$) for ceftobiprole and 0.88 ± 0.22 log$_{10}$ CFU/ml ($n = 28$) for vancomycin (Fig. 2). The slightly greater CFU reduction for vancomycin-treated rats was not significantly different ($P = 0.59$) from that for ceftobiprole-treated animals.

**Resistance testing.** The potential emergence of ceftobiprole-resistant mutants during therapy of chronic tissue cage infections by MRSA strain MRGR3 was studied. No isolates showing increased MIC towards the cephalosporin was observed for MRSA strain MRGR3 recovered from fluid samples obtained from 29 different tissue cages.

**DISCUSSION**

The ongoing search for new antistaphylococcal agents (1, 6, 30, 48) is justified by the continuing increase in nosocomial MRSA isolates displaying multidrug resistance and by the recent appearance and dissemination of distinct clones of community-acquired MRSA (8, 31). While some recently approved antimicrobials circumvent mechanisms conferring methicillin resistance (17), ceftobiprole directly targets PBP2, whose low affinity for marketed β-lactams is responsible for the methicillin resistance phenotype (22). The MIC of the MRSA strain MRGR3 used in our animal study is consistent with previously reported MIC$_{50}$ (≥2 μg/ml) and MIC$_{90}$ (≥4 μg/ml) values for ceftobiprole with diverse panels of MRSA clinical isolates (2, 14, 15, 18, 22, 26; L. M. Ednie and P. C. Appelbaum, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-2021, 2004; R. Reynolds, D. M. Livermore, and the BSAC Working Party on Bacteraemia Resistance Surveillance, 44th

Over the past decade, studies using the tissue cage model have confirmed the limited efficacy of antibiotic regimens for treatment of S. aureus foreign-body infections (7, 12, 13, 32, 35, 36, 43, 44). These studies indicated that bactericidal levels of anti-staphylococcal agents needed to be reached in tissue cage fluids to reduce viable counts of S. aureus in chronically infected tissue cages. A pharmacokinetic study of two dosing regimens of ceftobiprole medocaril identified 150 mg- eq. /kg of ceftobiprole/kg every 12 hours as promoting bactericidal levels of ceftobiprole in tissue cage fluids for at least 75% of the dosing interval.

At the selected doses ceftobiprole and vancomycin each achieved comparable peak and trough levels in tissue cage fluids. The two drugs also have comparable protein binding values of 38% and 30%, respectively (Basilea Pharmaceutica AG, data on file). We observed no significant difference in CFU reductions between ceftobiprole-treated and vancomycin-treated animals, though there was a significant reduction in viable counts of MRSA strain MRGR3 for antibiotic-treated relative to control animals. The modest reductions in viable counts observed after 7 days of treatment with high doses of ceftobiprole or vancomycin attests to the intractability of this infection model (7, 12, 32, 35, 43, 44).

While comparison between experimental therapy in animals and clinical use in humans should be done with caution, the following arguments indicate that the serum/plasma levels required to reach bactericidal levels of ceftobiprole in tissue cage fluids were not exceeding those reasonably attainable with human dosing. First, previous experimental studies have shown the efficacy of ceftobiprole to be directly related to the percentage of the dosing interval during which the concentration of ceftobiprole in plasma was above the minimum MIC of the target pathogen (T>MIC) (D. R. Andes and W. Craig, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1079, 2000). For MRSA infection, a conservative target of 4 μg/ml was retained. Second, the dosing regimen in the therapeutic phase 2 study in complicated skin and skin structure infection was 750 mg twice a day given as a 30-min infusion. At this dose the plasma levels of ceftobiprole in patients remained above the target value for 8 h (A. H. Schmitt-Hoffmann, M. Harsch, M. Heep, M. Schleimer, T. Brown, A. Man, and W. O’Riordan, Abstr. 14th ECCMID, abstract P-1031, 2004). Third, ceftobiprole in animals and humans is restricted to the extracellular compartment, with no species differences in the extent of plasma protein binding. When ceftobiprole was administered to rats at a dose of 150 mg/kg twice a day, the predicted plasma concentrations of the cephalosporin were well under 4 μg/ml at 6 h after administration, corresponding to a T>MIC of less than 50%. Since the dose given to rats was somewhat inferior to the human therapeutic dose in terms of T>MIC, the efficacy of this suboptimal dose in this stringent model should be considered predictive for the efficacy in human at therapeutic doses.

Of six different fluoroquinolones tested using regimens producing bactericidal levels in tissue cage fluids (7, 12, 32, 44), only temafloxacin (withdrawn by Abbott laboratories in 1002, four months after Food and Drug Administration approval, for reasons of toxicity) and levofloxacin led to significantly higher reductions in viable counts than vancomycin (7, 44), whereas high-dose regimens of ciprofloxacin or teicoplanin failed to reduce significantly viable counts of MRSA strain MRGR3 (7, 36). Not surprisingly, the in vivo activity of ceftobiprole was much better than that of imipenem (35), a carbapenem once considered as a possible treatment for MRSA infections (10).

An important outcome of this work was the demonstration of a complete lack of emergence of MRSA strain MRGR3 derivatives with diminished susceptibility to ceftobiprole, following 7 days of intensive therapy with this cephalosporin. This result is remarkable because the selective medium used for screening resistant subclones contained 4 μg/ml of cefto- prole, which is just the MBC of this cephalosporin for MRSA strain MRGR3. Such a low concentration of ceftobiprole should facilitate detection of colonies exhibiting marginally decreased susceptibility to this antimicrobial agent. Historical comparison with previous studies in our tissue cage model indicates that emergence of resistance or phenotypic tolerance occurred with a number of antimicrobial agents. For example, microcolonial subpopulations of MRGR3 highly resistant to imipenem were detected on posttherapy isolates on imipenem-supplemented agar plates after 72 h of growth at 37°C (35). In other studies from our group, the local environment of chronic MRSA tissue cage infections promoted the in vivo emergence of phenotypic tolerance (41) or resistance (36, 42) to glycopeptides, especially teicoplanin, in strain MRGR3. Finally, the absence of resistance development to ceftobiprole in MRSA strain MRGR3 also contrasts with the emergence of resistant subpopulations during daptomycin therapy of S. aureus-infected tissue cages (43).

Collectively, these data strongly suggest that the tissue cage model is adequate for detecting emergence of ceftobiprole-resistant subpopulations of S. aureus, in particular because cage fluid is a protein-rich (ca. 20 g/liter) exudate enriched in nutrients presumably released from lysed host cells (e.g., red blood cells, leukocytes, others), which supports bacterial growth much better than serum (49). Our results also confirm previous reports that staphyloccoci (9, 18, 22, 23; T. Bogdanovich, B. Bozdogan, and P. C. Appelbaum, Abstr. Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-2018, 2004) and other pathogens (27) are refractory to development of endogenous resistance to ceftobiprole. This likely represents a significant advantage of ceftobiprole over glycopeptide or lipopeptide therapy of foreign-body infections.

The favorable pharmacodynamic and therapeutic properties of ceftobiprole for the treatment of severe infections due to MRSA or other multiresistant gram-positive bacteria warrant further studies of this cephalosporin in clinical settings.

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