In Vitro Activities of a Novel Cephalosporin, CB-181963 (CAB-175), against Methicillin-Susceptible or -Resistant Staphylococcus aureus and Glycopeptide-Intermediate Susceptible Staphylococci

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We examined the activity of CB-181963, a novel cephalosporin, against methicillin-resistant Staphylococcus aureus (MRSA) (n = 200), methicillin-susceptible S. aureus (MSSA) (n = 50), glycopeptide-intermediate Staphylococcus species (GISS) (n = 47), and VRSA (n = 2) isolates. CB-181963 exhibited MIC profiles similar to those of linezolid against MRSA and GISS; however, activity against MSSA was similar to that of vancomycin. Time-kill study results of investigations of activity against MRSA, MSSA, and GISS at 24 h were as follows: CB-181963 activity = vancomycin activity > linezolid activity (P < 0.001); CB-181963 = quinupristin-dalfopristin = vancomycin > linezolid (P < 0.05); CB-181963 > linezolid (P = 0.003); and CB-181963 = quinupristin-dalfopristin = vancomycin. CB-181963 may provide an alternative treatment for multidrug-resistant staphylococci.

The incidence of methicillin-resistant Staphylococcus aureus (MRSA) has been increasing at an alarming rate since it was first reported in the 1960s (21). In 1996, the first report of a glycopeptide-intermediate susceptible S. aureus (GISA) was described in Japan (4). Since then, other cases of GISA infections have been reported worldwide (1, 2, 3, 4, 5, 8, 13, 16, 17, 20, 22, 24, 25, 27). These GISA strains are generally found in patients who have been exposed to long-term vancomycin therapy with drug MICs of >4 and <32 μg/ml. Heterogeneous GISA (hGISA) and heterogeneous glycopeptide-intermediate Staphylococcus species (hGISS) are defined as those for which vancomycin MICs are 1 to 4 μg/ml but which contain subpopulations that can grow on agar plates supplemented with vancomycin (4 μg/ml) (2). These strains were first described by Hiramatsu et al. and may be the first step in the development of GISA strains (2). Recently, the first two vancomycin-resistant S. aureus (VRSA) strains (from a Michigan and Pennsylvania patient, respectively) were reported in June and September of 2002, which increased the need to find alternative agents (6, 7).

The ideal cephalosporin compound against MRSA would combine the characteristics of high-level affinity for PBP 2a and stability against degradation by staphylococcal β-lactamase (9, 15, 18). This has been the driving factor for developing cephalosporins with activity against MRSA.

CB-181963 (Fig. 1) is a novel parenteral investigational cephalosporin belonging to the azomethine subclass of cephalosporins that has demonstrated in vitro bactericidal activity against a range of pathogens, including MRSA (Cubist Pharmaceuticals, Lexington, Mass.; D. Damphousse, D. Dvorchik, D. Benziger, and K. Galil, Abstr. 13th Eur. Cong. Clin. Microbiol. Infect. Dis., abstr. P792, 2003; and J. Silverman, N. Cronceo, V. Laganas, G. Thorne, J. Alder, Abstr. 13th Eur. Cong. Clin. Microbiol. Infect. Dis., abstr. P793, 2003). CB-181963 displays strong binding to altered PBP 2a (Silverman et al., 13th ECCMID, abstr. P793). In vitro, CB-181963 demonstrated marked efficacy against MRSA, Klebsiella pneumoniae, Streptococcus pneumoniae, and Escherichia coli (R. Cha, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. E2011, 2003; Silverman et al., 13th ECCMID, abstr. P793). CB-181963 was noted to have a spectrum and safety profile similar to that of ceftiraxone, with additional activity against MRSA and Enterococcus faecalis reported from human and animal studies completed thus far (Cubist Pharmaceuticals; Silverman et al., 13th ECCMID, abstr. P793). The compound pharmacokinetics from initial human experience suggested that CB-181963 has elimination half-life mean values of 1.61 to 1.68 h and would be administered parenterally in the range of 500 to 1,000 mg twice daily (Damphousse et al., Abstr. 13th ECCMID, abstr. P792). A dose of 500 mg results in a maximum concentration of drug in serum of 37.3 ± 1.6 mg/ml, and a dose of 1,000 mg results in a maximum concentration of drug in serum of 72.0 ± 14.8 mg/ml; serum was maintained above the MIC (Damphousse et al., Abstr. 13th ECCMID, abstr. P792).

The aim of this study was to evaluate the activity of CB-181963 against MRSA, MSSA, GISS, and VRSA compared to that of vancomycin, linezolid, and quinupristin-dalfopristin.

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There were a total of 297 clinical strains: 200 clinical strains of MRSA, 50 clinical strains of MSSA, and 47 clinical strains of...
GISS. GISS consisted of 35 strains of *S. aureus* (14 GISA strains [MIC = 8 μg/ml] and 21 hGISA strains [MIC = 4 μg/ml]), 8 strains of *Staphylococcus epidermidis* (6 GISS strains [MIC = 8 μg/ml] and 2 hGISS strains [MIC = 4 μg/ml]), and 4 strains of *Staphylococcus haemolyticus* (3 GISS strains [MIC = 8 μg/ml] and 1 hGISS strain [MIC = 4 μg/ml]). MRSA and MSSA strains were obtained from Detroit Medical Center, Detroit, Michigan, and William Beaumont Hospital, Royal Oak, Michigan. A total of 47 clinical strains of GISS were obtained from Keiichi Hiramatsu (Tokyo, Japan), the Centers for Disease Control and Prevention, the Detroit Medical Center, and the Network on Antimicrobial Resistance in *S. aureus* program. Two clinical strains of VRSA, including Michigan and Pennsylvania strains (VRSA-MI and VRSA-PA), were obtained from the Detroit Medical Center Laboratory from William J. Brown and from the Network on Antimicrobial Resistance in *S. aureus* program. ATCC 25923, a standard reference strain which is oxacillin susceptible, was used as a control.

CB-181963 (Cubist Pharmaceuticals) and quinupristin-dalfopristin (Aventis Pharmaceuticals Inc., Bridgewater, N.J.) were obtained from their respective manufacturers. Vancomycin analytical powder was commercially purchased from Sigma Chemical Company, St. Louis, Mo. Linezolid (Pharmacia, Kalamazoo, Mich.) was obtained commercially.

Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium (25 mg/liter) and magnesium (12.5 mg/liter) (SMHB) was used for all broth microdilution susceptibility testing and selective time-kill experiments. Tryptic soy agar (TSA; Difco Laboratories) for MRSA isolates and brain heart infusion (BHI) agar (Becton-Dickinson, Sparks, Md.) for GISS and VRSA isolates were used for bacterial quantification of samples from time-kill experiments.

MICs and minimum bactericidal concentrations (MBCs) were determined in duplicate using a microdilution technique in accordance with the National Committee for Clinical Laboratory Standards (23).

Selective time-kill experiments using four strains each of MRSA, MSSA, GISS, and VRSA were performed in triplicate. All drugs were tested at two to four times the MIC for MRSA, MSSA, GISS, and VRSA strains. However, against VRSA strains vancomycin was tested at a fixed concentration of 30 μg/ml. Three to five colonies from overnight growth on TSA at 37°C were added to normal saline and adjusted to produce a 0.5 McFarland standard suspension of organisms. This suspension was diluted with SMHB to achieve an inoculum of 10⁶ CFU/ml. A 0.2-ml suspension of each organism was added to 1.8 ml of SMHB with a 0.1-ml stock solution of each antibiotic in a 24-well tissue culture plate (final volume, 2.0 ml per well). Culture wells were incubated at 37°C with constant shaking for 24 h. Sample aliquots (0.1 ml) were removed from cultures at 0, 8, and 24 h. Antimicrobial carryover was minimized by serial dilution (10- to 10,000-fold) of plated samples in conjunction with vacuum filtration, in which samples were washed through a 0.45-μm-pore-size micron filter with normal saline. These filters were then plated onto TSA or BHI agar and incubated to confirm colony counts. Colony counts were determined by plating 50 μl of each diluted sample onto TSA and BHI plates with an automated spiral dispenser (WASP; Don Whitley Scientific Limited, West Yorkshire, England). We determined these methods to have a lower limit of reliable detection of 2.0 log₁₀ CFU/ml. Growth control wells for each organism were prepared without antibiotic and run in parallel to the antibiotic test wells. Bactericidal activity was defined as a reduction of 3 log₁₀ CFU/ml (99.9% killing) in bacterial density from the starting inoculum. Time to 99.9% killing was determined by linear regression of the sample points when r² ≥ 0.95 or by visual inspection.

All statistical analyses were performed using SPSS statistical software (release 11.5; SPSS, Inc. Chicago, Ill.). Colony counts at 24 h and time to 99.9% killing were compared between groups by use of one-way analysis of variance followed by

![FIG. 1. CB-181963 chemical structure.](http://aac.asm.org/Downloadedfromhttp://aac.asm.org)}
Tukey’s post hoc test for multiple comparisons. A $P$ value of $\leq 0.05$ indicated statistical significance.

The drug activities for the 200 clinical strains of MRSA, 50 clinical strains of MSSA, 47 clinical strains of GISS, and 2 clinical strains of VRSA are reported in Table 1. The levels of activity of CB-181963 against all clinical strains (MRSA, MSSA, GISS, and VRSA) indicated equivalent levels of potency. CB-181963 activity against MRSA and VRSA was equal to that of linezolid; CB-181963 activity levels were twofold higher against MSSA and twofold lower against GISS. CB-181963 was four- to eightfold less active than quinupristin-dalfopristin against MRSA, MSSA, GISS, and VRSA. However, quinupristin-dalfopristin was 2-, 16-, 128-, and 2,048-fold more active than vancomycin against MRSA and MSSA, GISS, VRSA-MI, and VRSA-PA, respectively. CB-181963 demonstrated similar levels of activity against MRSA and GISS strains, with significant activity against the MSSA strains. There were two clinical strains of GISS for which the CB-181963 MIC was 8 $\mu$g/ml. The MBC and MIC results demonstrated that CB-181963 possessed bactericidal activity against MRSA and VRSA. The time-kill mean results of the four randomly selected strains of MRSA, MSSA, and GISS strains are shown in Fig. 2. At concentrations four times the MIC, CB-181963 and vancomycin achieved 99.9% killing against MRSA in 8 h, which was higher than the level of killing observed with linezolid and
quinupristin-dalfopristin. The bactericidal activity of CB-191863 and vancomycin was maintained for up to 24 h. Against MRSA at 8 and 24 h, CB-181963 and vancomycin were significantly more active than linezolid ($P < 0.001$). Linezolid and quinupristin-dalfopristin activities against MRSA at 24 h were not significantly different. In addition, the relative bactericidal activity of quinupristin-dalfopristin against MRSA at 24 h was not significantly different from those of CB-181963 and vancomycin. CB-181963 and quinupristin-dalfopristin ($P < 0.001$) achieved 99.9% killing against MSSA in 8 h at concentrations four times the MIC, which was higher than the level of killing observed with linezolid and vancomycin. This bactericidal activity was maintained up to 24 h for CB-181963 and quinupristin-dalfopristin. CB-181963, quinupristin-dalfopristin, and vancomycin were significantly more active against MSSA than linezolid ($P < 0.05$) at 8 and 24 h. The activities of CB-181963, quinupristin-dalfopristin, and vancomycin against MSSA at 24 h were not significantly different. At concentrations two times the MIC, CB-181963 achieved 99.9% killing against GI58 at 24 h, which was higher than the level of killing observed with linezolid ($P = 0.003$). However, the activities of CB-181963, quinupristin-dalfopristin, and vancomycin were not significantly different at 24 h. CB-181963 and quinupristin-dalfopristin at two times the MIC demonstrated bactericidal activity against VRSA-MI at 24 h. However, only CB-181963 achieved 99.9% killing against VRSA-PA at 24 h, which was higher than the level of killing observed with quinupristindalfopristin and vancomycin ($P < 0.01$).

CB-181963 is a new parenteral cephalosporin with potent in vitro activity against a broad range of gram-positive pathogens, including methicillin-susceptible and -resistant S. aureus. The spectrum of activity extends to GI58 and VRSA as well as a variety of gram-negative pathogens. CB-181963 is one of a number of cephalosporins, such as Ro 63-9141 (BAL9141), RWJ-54428 (MC-02479), and S-3578, with various degrees of activity against MRSA (10, 11, 12, 14, 18, 19, 26, 28; Damp-housse et al., Abstr. 13th ECCMID, abstr. P792; Silverman et al., 13th ECCMID, abstr. P793).

The incidence of MRSA strains, as well as that of multidrug-resistant staphylococci, has greatly increased (21). Cephalosporins have been widely used in infection therapy because of their broad-spectrum antibacterial activity and lower frequency of side effects. However, their efficacy against MRSA has been insufficient to eradicate infections caused by the pathogens. Therefore, a search for cephalosporin derivatives with potent activity against MRSA is an option to be explored. CB-181963 has demonstrated potent in vitro activity against MRSA (Silverman et al., 13th ECCMID, abstr. P793). In the present study, the susceptibility of clinical staphylococcal isolates, including MRSA, MSSA, GI58, and VRSA isolates, was examined. Our data further established that CB-181963 has potent antistaphylococcal activity in vitro (D. C. Draghi, C. Thornsberry, D. F. Sahm, J. A. Karlowsky, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-544, 2003; Silverman et al., 13th ECCMID, abstr. P793). Although CB-181963 had a slightly higher MIC than vancomycin, the bactericidal activity was equal to and greater than that of vancomycin, linezolid, and quinupristin-dalfopristin against MRSA and GI58, respectively. These results indicate that CB-181963 is a novel cephalosporin derivative that demonstrates activity against susceptible and multidrug-resistant staphylococci. CB-181963 may provide an alternative option for the treatment of MRSA infections. However, further pharmacokinetic and pharmacodynamic, as well as clinical investigations, are warranted to determine its ultimate place in therapy versus presently available options.

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REFERENCES


