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Evaluation of CP-99,219, a New Fluoroquinolone, for Treatment of Experimental Penicillin- and Cephalosporin-Resistant Pneumococcal Meningitis

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CP-99,219 is a new fluoroquinolone that has excellent activity against gram-positive organisms including penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* strains. In our well-established rabbit model of meningitis, we conducted experiments to determine the concentrations of CP-99,219 in cerebrospinal fluid (CSF) after intravenous administration and its ability to eradicate two penicillin-resistant pneumococcal isolates. The peak and trough concentrations of CP-99,219 in the CSF were from 19 to 25% of the concentrations simultaneously obtained in serum and were unaffected by concomitant dexamethasone administration. Compared with untreated (control) animals, three doses of CP-99,219 given 5 h apart significantly reduced the bacterial count in CSF by 5 to 6 $\log_{10}$ CFU at 10 h. Although 47% of the dexamethasone-treated animals and 18% of those not given the steroid had positive cultures at 24 h (14 h after administration of the last antibiotic dose), the mean bacterial counts did not change from those observed at 10 h. Additionally, only results for animals infected with one of the two pneumococcal strains appeared to be affected by concomitant dexamethasone therapy.

CP-99,219, a new investigational fluoroquinolone, is an azabicyclo-naphthyridone that has excellent in vitro activity against several organisms such as penicillin-resistant pneumococci and vancomycin-resistant enterococci (1, 5, 8). Because of the increase in penicillin- and cephalosporin-resistant pneumococcal isolates worldwide, new antibiotics with improved activities against these organisms are needed, especially to treat infections in which the level of penetration of many antibiotics is reduced, such as in acute otitis media and bacterial meningitis.

We conducted experiments in a pneumococcal meningitis model to determine the penetration of CP-99,219 into cerebrospinal fluid (CSF), the effect of dexamethasone therapy on the concentrations of this drug in CSF, and the bacteriologic effect of CP-99,219 against two penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* strains.

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MATERIALS AND METHODS

***S. pneumoniae* strains.** Two pneumococcal isolates from children with meningitis who failed standard therapy were used (6, 7). The inoculum was prepared as described previously (14). All experiments with these pneumococcus-resistant strains were conducted during a 6-month period in which each study evaluating an antimicrobial agent had at least two untreated control animals to be certain that the virulence of the organism had not changed, and the inocula in all the experiments ranged from 9×10^4 to 4×10^5/ml.

Experimental meningitis model. We used our well-characterized meningitis model (9, 10, 12, 18), modified from the original description of Dacey and Sande (2). CSF samples (100 to 200 μl) were withdrawn before and at 5, 10, and 24 h after the antibiotic was given. After dilution of CSF 10-fold in phosphate-buffered saline, bacterial concentrations were quantified by plating 100 μl of the mixture onto blood agar plates and incubating the plates for 24 h in 5% CO_2 at 35°C. The lower limit of detection was 10 CFU/ml. For purposes of statistical analysis, a value of 1 CFU/ml (0 $\log_{10}$ unit) was assigned to the negative cultures. The remaining CSF was centrifuged, and the supernatant was stored at −70°C for determination of antibiotic concentrations. Additional CSF and blood samples were obtained at 1 h and at 0.5, 1, 2, and 5 h, respectively, after treatment for measurement of antibiotic concentrations. These samples were stored as described above.

Therapy. The rabbits were treated with 25 mg of CP-116,517 (activity, 80.2%; prodrug of CP-99,219; Pfizer Central Research, Groton, Conn.) per kg of body weight at 0, 5, and 10 h. After initial dose ranging studies, this dosage was chosen because it achieved concentrations in serum similar to those in the sera of adults who participated in phase I studies (15a). Some animals received dexamethasone (Luitpold Pharmaceutical Inc., Shirley, N.Y.) intravenously at 1 mg/kg at 0 and 10 h.

Antibiotic concentration determination. CP-99,219 concentrations in CSF and serum were measured by disk diffusion microbioassay with *Bacillus subtilis* as described elsewhere (17). The lower limits of detection were 0.08 and 0.1 μg/ml in CSF and serum, respectively. The interassay coefficients of variation were 2.5 and 4% for concentrations in serum and CSF, respectively, and the intraassay coefficients of variation were 5.5 and 6.3% for concentrations in serum and CSF, respectively. The serum concentration-time curves for each subject were analyzed by nonlinear least-square regression analysis with the AUTOAN program (11).

Statistical analysis. The Student *t* test and one-way analysis of variance (Newman-Keuls multiple comparisons test) were used for parametric data. Comparison of positive and negative cultures were analyzed by the two-tailed Fisher exact test. A P value of <0.05 was considered significant.

RESULTS

The MICs and MBCs of penicillin, ceftriaxone, vancomycin, and CP-99,219 for the two strains used in these experiments are given in Table 1.

The antibiotic concentrations in serum and CSF are presented in Fig. 1. The half-life of CP-99,219 in serum was 68 to 72 min. Dexamethasone therapy did not affect the CSF CP-99,219 concentrations, which were comparable in both treatment groups. The concentrations of CP-99,219 in CSF were approximately 19 to 25% of the concomitant concentrations in serum and were unaffected by dexamethasone administration.

The clearance of *S. pneumoniae* from the CSF was similar in

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TABLE 1. MICs and MBCs for the strains used in the experiments

Antibiotic	JM		JG	
	MIC (μg/ml)[a]	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
Penicillin	1	1	2	2
Ceftriaxone	1	1	4	4
Vancomycin	0.25	0.5	0.25	0.25
CP-99,219	0.06	0.125	0.06	0.125

[a] By the microdilution method with Mueller-Hinton broth supplemented with 3 to 5% lysed horse blood.

the dexamethasone- and non-dexamethasone-treated rabbits, whether the JM strain (which was more susceptible to penicillin) or the JG strain (which was more resistant to penicillin) was inoculated (Fig. 2). With the exception of cultures at time zero, the numbers of CFU of *S. pneumoniae* in CSF were higher in animals inoculated with JM than in those inoculated with JG; however, the differences were not statistically significant. Although the mean bacterial counts were similar at 10 and 24 h for dexamethasone- and non-dexamethasone-treated rabbits, the numbers of positive cultures at 10 and 24 h were higher for the dexamethasone-treated animals (7 versus 4 at 10 h and 8 versus 3 at 24 h for dexamethasone- versus non-dexamethasone-treated animals, respectively). There were no significant differences between the groups. This experiment has a power of 80% to detect a difference in proportions of 0.44.

The bacteriologic effects of CP-99,219, vancomycin, and ceftriaxone (JM-infected animals only) therapy were compared to determine whether concomitant dexamethasone therapy affected the response. Against the JM strain (Table 2), vancomycin and CP-99,219 were most active, as judged by the change in bacterial concentrations 10 and 24 h after the start of therapy. Dexamethasone therapy did not significantly reduce the bacteriologic response of any of the three antibiotics, although the change in the $\log_{10}$ CFU was smaller when dexamethasone was given with vancomycin at 10 h and with all three antibiotics at 24 h.

Against the more penicillin-resistant JG strain (Table 3), only vancomycin and CP-99,219 were assessed because ceftriaxone was consistently ineffective in this model. Dexamethasone therapy did not alter the bacteriologic responses of these two antibiotics.

DISCUSSION

CP-99,219 has superior activity against susceptible and multiple drug-resistant *S. pneumoniae* strains compared with those of other quinolones (1, 5, 8, 15). As a result, this antibiotic could potentially be used to treat patients with meningitis caused by penicillin- and cephalosporin-resistant pneumococcal strains. The efficacy of CP-99,219 in these experiments was at least comparable to that of vancomycin therapy against the two strains used and to that of ceftriaxone against the JM strain.

Because dexamethasone can reduce the level of penetration of antibiotics into the CSF and can potentially delay sterilization of CSF cultures, as suggested by our previous studies of vancomycin in experimental meningitis caused by resistant pneumococcal strains (14), we were interested to determine whether dexamethasone had any effect on the penetration and activity of CP-99,219 in the same animal model. The mean concentrations of CP-99,219 in CSF were not affected by dexamethasone administration (4). Although, the bacterial concentrations in CSF after the start of CP-99,219 treatment were equivalent in dexamethasone- and non-dexamethasone-treated animals, there were more positive cultures at 10 and 24 h in JM-infected rabbits receiving steroids. The clinical significance of these observations is unknown. The number of animals studied was relatively small ($n = 17$); thus, to have demonstrated a statistically significant difference, assuming an α value of 0.05 and an 80% power to detect a decrease in bacterial concentration, it would have been necessary to include 47

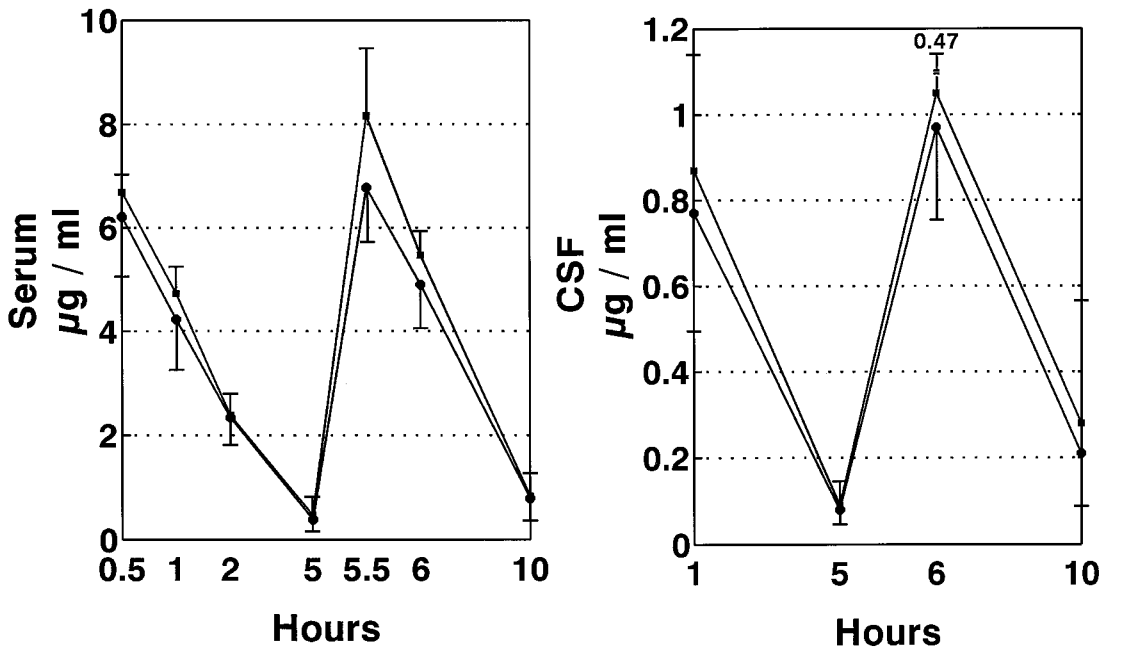

FIG. 1. CP-99,219 concentrations in the serum and CSF of rabbits infected with penicillin- and cephalosporin-resistant strains of *S. pneumoniae* and treated with CP-99,219 with (■) or without (●) dexamethasone. There were no significant differences.

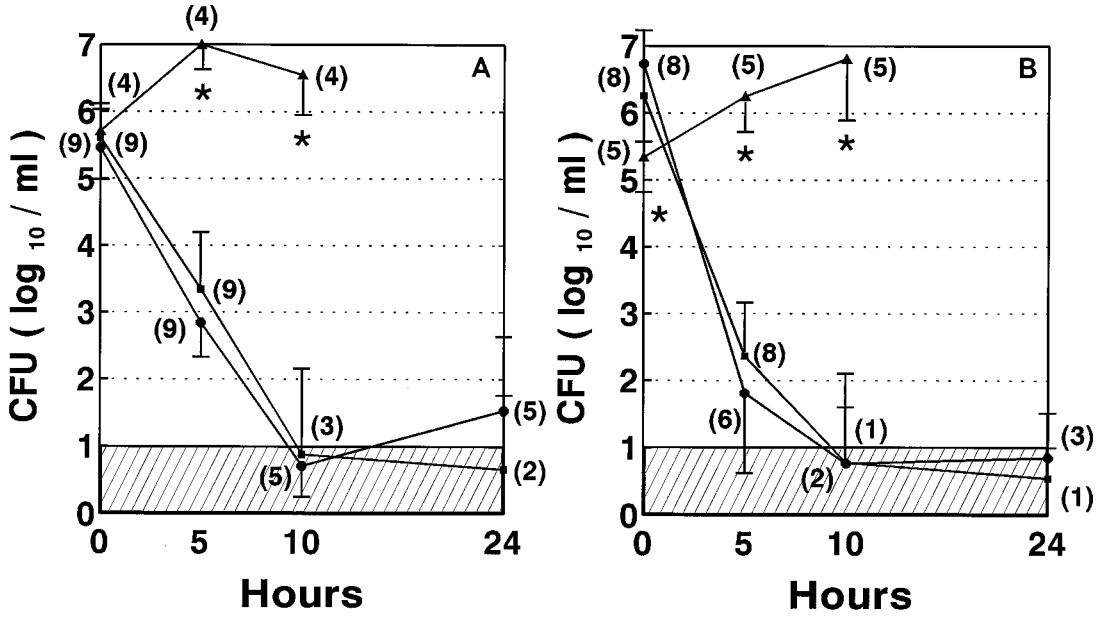

FIG. 2. Mean ± standard deviation bacterial concentrations in CSF and number of positive cultures (in parentheses) after intracisternal inoculation of an intermediately penicillin-susceptible strain (JM) (A) and a highly penicillin-resistant strain (JG) (B). Rabbits were treated with CP-99,219, either alone or with dexamethasone, or were not treated (control). All of the animals included in the treatment groups survived the 36 to 38 h of these experiments. The lower limit of detection of bacteria in CFU was 1 $\log_{10}$ CFU/ml (shaded area). *, $P < 0.05$ for treated versus untreated (control) rabbits. ■, rabbits treated with CP-99,219; ●, rabbits treated with CP-99,219 and dexamethasone; ▲, nontreated (control) rabbits.

animals in each study group (dexamethasone- and non-dexamethasone-treated rabbits). Furthermore, direct clinical application of data derived from this experimental meningitis model is problematic. Rather, we believe that data derived from this model provide a framework for considering evaluation of an antimicrobial agent in humans. As a rule, antibiotics that achieve adequate activity in CSF and satisfactory reduction in bacterial density in CSF after intravenous administration to rabbits with experimental meningitis have been effective for therapy of meningitis in infants and children (3, 4, 13, 16, 19).

We believe that initial, empiric therapy for meningitis in areas with a high prevalence of penicillin- and cephalosporin-resistant *S. pneumoniae* strains should include two antibiotics: either ceftriaxone or cefotaxime plus vancomycin if dexamethasone is not given, or rifampin when dexamethasone is used, until the results of susceptibility studies are available. Because of its activity against resistant pneumococci and adequate penetration into CSF in experimental meningitis, CP-99,219 may represent an alternative agent for initial, empiric treatment in humans with bacterial meningitis. Additional studies of CP-99,219 are required to define its clinical pharmacokinetics, safety, and efficacy in infants and children before this drug can be considered for use.

TABLE 2. Comparison of bacterial concentrations in CSF of rabbits treated with vancomycin, ceftriaxone, or CP-99,219 with or without dexamethasone or with no therapy (control group) after intracisternal inoculation of strain JM[a]

Treatment group[b]	Bacteriologic response (mean change in $\log_{10}$ CFU) at the following times after the start of therapy[c]:		
	0–5 h (*A*)	0–10 h (*B*)	0–24 h (*C*)
1. Control	+1.3	+1.0	ND[d]
2. Van	−4.0	−5.5	−6.1
3. Van + DXM	−2.7	−3.9	−3.7
4. CRO	−2.7	−2.9	−5.6
5. CRO + DXM	−1.9	−2.2	−2.6
6. CP-99,219	−2.3	−4.7	−5.0
7. CP-99,219 + DXM	−2.6	−4.8	−3.9

[a] Penicillin and ceftriaxone MICs for strain JM were 1 and 2 μg/ml, respectively. Data for treatment groups 2 through 5 are from reference 14.
[b] Van, vancomycin; DXM, dexamethasone; CRO, ceftriaxone.
[c] Significant differences ($P < 0.05$): *A*, groups 2 to 7 versus group 1; *B*, groups 2 to 7 versus group 1, group 2 versus groups 4 and 5, and groups 6 and 7 versus group 5; *C*, group 2 versus group 5.
[d] ND, not done; death occurred in most animals at between 10 and 24 h.

TABLE 3. Comparison of bacterial concentrations in CSF of rabbits treated with vancomycin or CP-99,219 with or without dexamethasone or with no therapy (control group) after intracisternal inoculation of strain JG[a]

Treatment group[b]	Bacteriologic response (mean change in $\log_{10}$ CFU) at the following times after the start of therapy[c]:		
	0–5 h (*A*)	0–10 h (*B*)	0–24 h (*C*)
1. Control	+0.9	+1.5	ND[d]
2. Van	−2.9	−2.5	−5.8
3. Van + DXM	−2.4	−3.9	−5.1
4. CP-99,219	−4.2	−5.9	−6.1
5. CP-99,219 + DXM	−5.2	−6.3	−6.3

[a] Penicillin and ceftriaxone MICs for strain JG were 2 and 4 μg/ml, respectively. Data for treatment groups 2 and 3 are from reference 14.
[b] Van, vancomycin; DXM, dexamethasone.
[c] Significant differences ($P < 0.05$): *A*, groups 2 to 5 versus group 1, group 5 versus groups 2 and 3, and group 4 versus group 2; *B*, groups 2 to 5 versus group 1 and group 5 versus group 3; *C*, no significant differences.
[d] ND, not done; death occurred in most animals at between 10 and 24 h.

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