

Bactericidal Activity of Cefoperazone with CP-45,899 Against Large Inocula of β -Lactamase-Producing *Haemophilus influenzae*

PAULINE K. W. YU AND JOHN A. WASHINGTON II*

Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

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Bactericidal activity of cefoperazone, alone and in combination with the β -lactamase inhibitor CP-45,899, was tested against inocula of 10^7 colony-forming units of β -lactamase-producing isolates of *Haemophilus influenzae* type b per ml. Of 19 strains tested, 10 required ≥ 64 μ g of cefoperazone per ml for killing, whereas no strains were killed by < 64 μ g of CP-45,899 per ml. Synergy occurred with the combination of 4 μ g of each agent per ml against 9 of the 10 cefoperazone-resistant strains.

The activity of commercially available cephalosporins against β -lactamase-producing isolates of *Haemophilus influenzae* is highly inoculum dependent (4-7, 10, 16, 17, 19), especially when bactericidal activity is tested (5). For this reason and because the numbers of colony-forming units (CFU) of *H. influenzae* per milliliter of cerebrospinal fluid (9) often far exceed those used for in vitro susceptibility testing, it has been suggested that inoculum effect be one of the variables examined when evaluating the organism's antimicrobial susceptibility (5, 16).

Previous studies in this laboratory (5) and by others (2, 12, 13) have shown marked inhibitory activity against low and high inocula of β -lactamase-producing strains of *H. influenzae* by cephalosporins which are stable in the presence of β -lactamases produced by a variety of gram-negative bacilli; however, our studies indicated that the bactericidal activity of cefoperazone was significantly diminished against high inocula of such strains (5).

Cefoperazone has been shown by Mitsuhashi et al. (12) to be stable to types I, II, III, and IV plasmid-mediated penicillinases and to be 10 to 10,000 times as stable as cephaloridine to cephalosporinases produced by *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Citrobacter freundii*. Neu et al. (13), however, demonstrated that cefoperazone was hydrolyzed by constitutive *E. coli* β -lactamases having cephalosporinase and penicillinase activities.

For these reasons, it was of interest to determine whether the bactericidal activity of cefoperazone against high inocula of β -lactamase-producing strains of *H. influenzae* would be restored in the presence of a β -lactamase inhib-

itor, CP-45,899 (penicillanic acid, 1,1-dioxide) (8).

MATERIALS AND METHODS

Nineteen of the previously studied β -lactamase-producing strains of *H. influenzae* type b (5) were selected for bactericidal testing at high inocula with cefoperazone and CP-45,899. β -Lactamase production was confirmed by the rapid chromogenic cephalosporin method (Nitrocefim; Glaxo Ltd., Greenford, Middlesex, England) (1). Minimal bactericidal concentrations (MBCs) were determined by a macro-broth dilution method. Dilutions of cefoperazone and CP-45,899 were prepared in Schaedler broth supplemented with 5% Fildes extract (peptic digest of blood). Isolates of *H. influenzae* were grown in Schaedler broth supplemented with 5% Fildes extract for approximately 6 h to a density equivalent to the turbidity of a McFarland no. 1 barium sulfate standard (approximately 10^8 CFU per ml) and diluted 1:10 before adding 0.5 ml (10^7 CFU/ml) to each tube containing 0.5 ml of cefoperazone or CP-45,899. Actual colony counts were determined by spreading 100 μ l of a 10^{-5} dilution of the inoculum onto two chocolate blood agar plates which were incubated for 48 h at 35°C. After 20 to 22 h of incubation at 35°C, 10- μ l samples were removed and spread over the surface of chocolate blood agar which was incubated at 35°C for 3 days. The MBC was the lowest concentration of drug which yielded 99.9% killing.

The bactericidal effects of cefoperazone and CP-45,899 in combination were studied with strains which had cefoperazone MBCs of ≥ 64 μ g/ml by testing multiple combinations of both drugs against inocula of 10^7 CFU/ml and determining, as described above, the lowest concentrations of each which in combination resulted in 99.9% killing. (The final volume of drug and inoculum in each tube was 2 ml.) The results of the combination studies were expressed as isobolograms (1).

Cefoperazone sodium powder and CP-45,899 pow-

der were provided for this study through the courtesy of A. K. Knirsch, Pfizer Central Research, Groton, Conn.

RESULTS

With inocula of 10^7 CFU/ml, 10 of the 19 strains had cefoperazone MBCs of ≥ 64 $\mu\text{g/ml}$ (Fig. 1). The MBCs of the remaining nine strains were 16 (two strains), 8 (two strains), 4 (two strains), 2 (two strains), and 0.5 $\mu\text{g/ml}$ (one strain). The MBCs of CP-45,899 were ≥ 64 $\mu\text{g/ml}$ in all instances. Of the 10 strains studied with combinations of both drugs, 9 displayed synergy and 1 did not. A combination of 4 μg each of cefoperazone and CP-45,899 per ml was bactericidal to the nine strains displaying synergistic effects (Fig. 1).

DISCUSSION

Ampicillin resistance by *H. influenzae* is R plasmid mediated by a constitutive β -lactamase (TEM-1) which is most active against benzylpenicillin, ampicillin, and cephaloridine (11, 15). Neu et al. (13) reported that a constitutive β -lactamase, which was derived from a strain of *E. coli* and which had penicillinase and cephalosporinase activity (probably the TEM-1 enzyme), hydrolyzed cefoperazone at a rate exceeding that of cephaloridine.

CP-45,899, an irreversible inhibitor of several bacterial penicillinases and cephalosporinases (8), has been found to enhance the inhibitory and bactericidal activity of ampicillin against β -lactamase-producing strains of *H. influenzae* (14, 18). CP-45,899 similarly enhanced the bactericidal activity of cefoperazone against β -lactamase-producing strains of *H. influenzae* in our study.

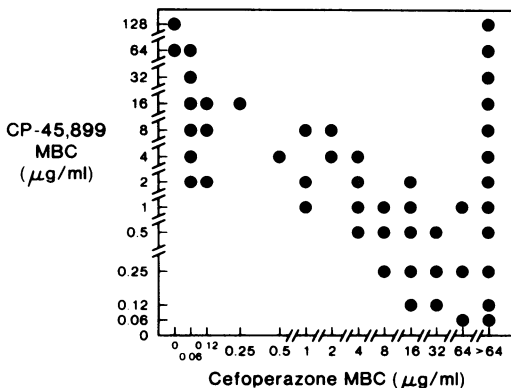


FIG. 1. Bactericidal activity of cefoperazone, alone and in combination with CP-45,899, against inocula of 10^7 CFU of 10 β -lactamase-producing, cefoperazone-resistant isolates of *H. influenzae* per ml.

Precisely what clinical significance these data have obviously remains speculative. Nonetheless, certain points bear emphasis. First, the number of CFU of *H. influenzae* per ml of cerebrospinal fluid is not infrequently 10^8 or greater (9). Second, cefoperazone MBCs against inocula of 10^7 CFU of the 19 β -lactamase-producing strains of *H. influenzae* per ml included in this study were ≥ 64 $\mu\text{g/ml}$. Third, studies in experimental rabbit models of bacterial meningitis have shown that concentrations of cefoperazone in cerebrospinal fluid are approximately 4 to 8% of those in serum (G. M. Allegro, M. A. Sande, and W. M. Scheld, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 560, 1980; U. B. Schaad and G. H. McCracken, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 563, 1980; J. R. Perfect and D. T. Durack, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 567, 1980). Fourth, central nervous system concentrations of antibiotics which are lower than the MBC of the infecting strain may be insufficient to guarantee a successful outcome (3). Unless, therefore, CP-45,899 penetrates the central nervous system in sufficient quantity to exert a synergistic effect on the bactericidal activity of cefoperazone against large numbers of β -lactamase-producing strains of *H. influenzae*, there probably should be serious reservations about the use of cefoperazone alone in the treatment of bacterial meningitis.

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