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Comparative Activity of Cefepime, Alone and in Combination, against Clinical Isolates of *Pseudomonas aeruginosa* and *Pseudomonas cepacia* from Cystic Fibrosis Patients

JOHN A. BOSSO,[1,2]* BARBARA A. SAXON,[3] AND JOHN M. MATSEN[3,4]

College of Pharmacy, University of Houston, 1441 Moursund Street,[1] and Department of Pediatrics, Baylor College of Medicine,[2] Houston, Texas 77030, and Department of Pathology[3] and Department of Pediatrics,[4] School of Medicine, University of Utah, Salt Lake City, Utah 84132*

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The comparative in vitro activity and synergy of cefepime were evaluated with clinical isolates of *Pseudomonas aeruginosa* and *Pseudomonas cepacia* from cystic fibrosis patients. The activity of cefepime, both alone and in combination, was comparable to those of other antibiotics. The clinical efficacy of cefepime in cystic fibrosis patients merits investigation.

Cefepime is a new, broad-spectrum cephalosporin antibiotic with pronounced activity against many gram-negative pathogens (2, 6, 7). It is more active than some of the currently marketed broad-spectrum cephalosporins against *Pseudomonas aeruginosa* and is stable against hydrolysis by common β-lactamases. Moreover, it has been found to be active against most gram-negative bacteria, including *P. aeruginosa*, that have developed resistance to other broad-spectrum cephalosporins (3). The objectives of the present study were to assess the comparative in vitro activity of cefepime against clinical isolates of *P. aeruginosa* and *Pseudomonas cepacia* obtained from cystic fibrosis patients and to determine the frequency of in vitro synergy in combinations of cefepime with other antipseudomonal agents against these same pseudomonal species.

Clinical isolates of *P. aeruginosa* ($n = 100$) and *P. cepacia* ($n = 25$) cultured from the sputa of cystic fibrosis patients and identified by standard microbiological methods were selected for the determination of the comparative activity of cefepime. Multiple isolates from the same patient were differentiated on the basis of colony morphology and antibiograms. The activity of cefepime was compared with those of ciprofloxacin, aztreonam, azlocillin, tobramycin, and ceftazidime. The MIC of each antibiotic was determined for all test isolates by microbroth dilution testing. Antibiotic reference powders were supplied as follows: cefepime, Bristol-Myers, Syracuse, N.Y.; ciprofloxacin and azlocillin, Miles Pharmaceuticals, West Haven, Conn.; aztreonam, E. R. Squibb, Princeton, N.J.; and tobramycin, Eli Lilly & Co., Indianapolis, Ind. Stock solutions were prepared in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (8). Mueller-Hinton broth supplemented with calcium and magnesium (final concentrations, 50 and 25 μg/ml, respectively) was diluted with the appropriate antibiotic concentration to provide twofold dilutions from 128 to 0.125 μg/ml. Microtiter plates were stored at −20°C and used within 30 days. Microtiter wells were inoculated with an actively growing inoculum adjusted to a 0.5 McFarland standard and further diluted to yield a final concentration of approximately 5×10^5 CFU/ml in the microtiter wells. The MIC was defined as the lowest concentration of drug that allowed no visible growth after 18 h of incubation at 35°C. Control organisms *Escherichia coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were included in all sets of inoculations. The results were considered valid if the MICs for the control organisms were within one twofold dilution of established values. Standard susceptibility and resistance breakpoints were used (8). The antibiotic concentrations inhibiting the growth of 50 and 90% of isolates (MIC_{50} and MIC_{90}, respectively) and the percentage of isolates susceptible to each antibiotic were determined. In addition, the extent of activity of cefepime against isolates not susceptible to ceftazidime, tobramycin, and ciprofloxacin was assessed.

Isolates of *P. aeruginosa* ($n = 100$) and *P. cepacia* ($n = 20$) also derived from cystic fibrosis patient sputa and identified by standard methods were used to study synergy (most, but not all, were also used in the comparative activity component of this study). Two-drug combinations consisting of cefepime and ciprofloxacin, tobramycin, and aztreonam were evaluated. Synergy was determined by the standard checkerboard technique. MICs were determined by microbroth dilution testing as described above but with antibiotic concentrations ranging from 1,024 to 0.062 μg/ml. Ten microliters of a 1:100 dilution of bacterial broth was added to each microdilution well, which contained 100 μl of antibiotic solution, such that a final inoculum of 5×10^5 μg/ml was produced. Microtiter plates were sealed in plastic bags and incubated overnight at 35°C. Synergy was defined as a fourfold or greater decrease in the MICs of both antibiotics (i.e., a cumulative fractional inhibitory concentration index of ≤0.5). Antagonism was defined as a fourfold or greater increase in the MIC of either antibiotic (i.e., a cumulative fractional inhibitory index of >4). Thus, the percentage of isolates of each species affected synergistically by each antibiotic combination was determined. The rate of synergy with organisms resistant to one or both antibiotics in any given combination was also determined.

The comparative in vitro activity of cefepime against *P. aeruginosa* and *P. cepacia* is presented in Table 1. The MIC_{90} of cefepime against *P. aeruginosa* was 16 μg/ml or one dilution higher than that of ceftazidime. However, the percentages of susceptible isolates were very similar for cefepime, ceftazidime, azlocillin, and tobramycin. Cefepime was active against 2 (22.2%) of 9 ceftazidime-resistant or

* Corresponding author.

TABLE 1. In vitro activities of cefepime and other antibiotics against 101 *P. aeruginosa* and 25 *P. cepacia* isolates from cystic fibrosis patients

Organism	Drug (breakpoint, μl/ml)	MIC (μg/ml)		% Susceptible
		50%	90%	
P. aeruginosa	Cefepine (≤8)	4	16	87.1
	Ceftazidime (≤8)	1	8	91.1
	Azlocillin (≤64)	8	128	87.1
	Aztreonam (≤8)	4	64	70.3
	Ciprofloxacin (≤1)	0.25	4	79.2
	Tobramycin (≤4)	1	4	91.1
P. cepacia	Cefepime (≤8)	16	>128	40
	Ceftazidime (≤8)	2	>128	68
	Azlocillin (≤64)	64	>128	52
	Aztreonam (≤8)	128	>128	8
	Ciprofloxacin (≤1)	4	8	28
	Tobramycin (≤4)	32	128	16

intermediate isolates, 16 (76.2%) of 21 of ciprofloxacin-resistant or intermediate isolates, and 6 (66.7%) of 9 tobramycin-resistant or intermediate isolates. Cefepime exhibited less activity against *P. cepacia* isolates, with only 40% being susceptible. While this percentage was higher than those observed for ciprofloxacin, tobramycin, and aztreonam, it was considerably lower than that observed for ceftazidime (68%). Cefepime was not active against any ceftazidime-resistant or intermediate *P. cepacia* isolate.

Rates of in vitro synergy are presented in Table 2. While some synergy occurred with each combination, there were few cases in which an isolate was resistant to one or both antibiotics but in which the synergistic MICs of both agents were within the susceptibility ranges. No cases of antagonism were observed with any combination.

The newer beta-lactams, aztreonam and ceftazidime, have proven useful in cystic fibrosis patients infected with *P. aeruginosa* (1, 5, 9, 10). Their efficacy in monodrug therapy has contributed to patient convenience and greater ease of home antibiotic therapy. On the basis of the results of this study, cefepime may also fit into this category. Its spectrum and degree of activity are very similar to those of ceftazidime. Fung-Tomc et al. reported a low rate of selection of resistant *P. aeruginosa* mutants by cefepime, lower than that by ceftazidime (4). In addition, cefepime resistance in ceftazidime- or cefotaxime-resistant *P. aeruginosa* mutants was rare. In contrast, we observed a low degree of activity of cefepime against ceftazidime-resistant isolates. The reasons for this discrepancy are unclear.

TABLE 2. In vitro synergy between cefepime and other antibiotics against isolates of *P. aeruginosa* and *P. cepacia* from cystic fibrosis patients

Combination	% Synergy[a] against:	
	P. aeruginosa (n = 100)	*P. cepacia* (n = 20)
Cefepime-ciprofloxacin	29 (7)	60 (25)
Cefepime-tobramycin	26 (9)	15 (10)
Cefepime-aztreonam	44 (9)	25 (10)

[a] Numbers in parentheses represent percentages of cases in which an isolate was resistant to one or both antibiotics but in which the synergistic MICs of both agents were within the susceptibility ranges.

Taken as a whole, the results of this study and other studies support the further evaluation of cefepime for *P. aeruginosa* infections in cystic fibrosis patients. The efficacy and safety of cefepime, both alone and in combination with other antibiotics, for pulmonary exacerbations of cystic fibrosis associated with *P. aeruginosa* should be evaluated and compared with those of currently used antibiotic regimens.

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