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In Vitro Activity and β-Lactamase Stability of Cefazaflur Compared with Those of β-Lactamase-Stable Cephalosporins

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The in vitro activity of cefazaflur, a parenteral cephalosporin, was determined against 590 clinical isolates. Cefazaflur inhibited the majority of gram-positive cocci at concentrations below 1 μg/ml except for enterococci. The agent was as active as cefamandole or cefoxitin against most *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis.* Although it inhibited a number of strains of *Enterobacter,* indole-positive *Proteus,* and *Serratia* resistant to cephalothin, it was much less active against these organisms than were cefamandole or cefoxitin.

Cephalosporin antibiotics have become increasingly important to the clinician as the spectrum of hospital infections has changed. New cephalosporins such as cefamandole and cefuroxime, and cefoxitin, a cephamycin, have significantly extended the spectrum of usefulness of agents in this class (6–9). Cefazaflur has been reported to be an agent with greater in vitro activity than cephalothin and cefazolin (1). We wished to compare the activity of this agent to these new compounds and to determine its β-lactamase stability.

MATERIALS AND METHODS

Cefazaflur was supplied by Smith, Kline, and French Laboratories. The other agents were gifts of their respective manufacturers. Fresh antibiotic dilutions were made daily in sterile broth. Bacterial strains tested were recent clinical isolates from patients hospitalized at the Columbia-Presbyterian Medical Center. Antimicrobial activity was measured by agar dilution using Mueller-Hinton agar (Baltimore Biological Laboratory [BBL]). Serial twofold dilutions of antibiotic were prepared, and an overnight culture of bacteria (inoculum 10^5 colony-forming units [CFU]) was applied as a drop with an inoculating device. Organisms were incubated at 35°C for 18 h. The minimal inhibitory concentration (MIC) of an antibiotic was defined as the lowest concentration that inhibited development of visible growth on agar. Activity against anaerobic bacteria was determined on Mueller-Hinton agar supplemented with sheep blood and with vitamin K. Plates were incubated at 35°C for 48 h in GasPak jars (BBL). *Haemophilus* and *Neisseria* were tested by using chocolate agar, which was incubated in the presence of 5% CO_2.

β-Lactamase assays. The enzymes were classified by the schema of Richmond (11). The β-lactamase assay procedures used were either the microiodometric method of Novick (9) or the spectrophotometric method (4). β-Lactamase was detected in clinical isolates by the chromogenic technique, using the Glaxo cephalosporin 87/132 (8).

β-Lactamase assays were performed by using purified (3) or partially purified enzymes which had been prepared by sonic disruption of bacteria followed by centrifugation to remove debris and chromatography on Sephadex G50.

RESULTS

The overall activity of cefazaflur against 590 gram-positive and gram-negative bacteria is given in Table 1. It inhibited the majority of gram-positive cocci at concentrations below 1 μg/ml, with the exception of *S. faecalis.* Some methicillin-resistant *S. epidermidis* were resistant to cefazaflur. *Neisseria* and *Haemophilus* were inhibited at concentrations below 1 μg/ml. This included three β-lactamase-producing isolates of both species. Overall activity against the members of the *Enterobacteriaceae* was good with more than 85% of the *E. coli, K. pneumoniae,* and *P. mirabilis* inhibited by concentrations of 12.5 μg or less per ml. The activity of the compound against other organisms such as *Enterobacter, Citrobacter,* and the indole-positive *Proteus* covered a wide range, with the mode inhibitory concentration nearly 100 μg/ml. Cefazaflur did not inhibit *Pseudomonas,* and high concentrations were required to inhibit *Bacteroides fragilis.*

The activity of cefazaflur was compared to that of cephalothin, cefamandole and cefoxitin (Table 2). Cefazaflur had activity against *S. aureus* comparable to that of cefamandole and superior to that of cefoxitin. Against *E. coli,* cefazaflur was as active as cefamandole and cefoxitin and superior to cephalothin. *Klebsiella*

were more susceptible to the cefazaflur than to the other agents, but it was less active against *Enterobacter* and *Citrobacter* than was cefamandole. Cefoxitin was more active than cefazaflur against indole-positive *Proteus, Providencia, Serratia,* and *Bacteroides.* Cefamandole was more active against these organisms than was cefazaflur. Cefazaflur had activity comparable to cefamandole and cefoxitin against *Salmonella.*

TABLE 1. *Overall in vitro activity of cefazaflur*

Organism	No. tested	MIC (μg/ml)	
		Range	Mode
S. aureus	29	0.1–12.5	0.2
S. epidermidis	30	0.2–200	0.6
S. pyogenes	12	0.1–0.6	0.2
S. agalactiae	22	<0.1–3.1	0.1
S. viridans	11	<0.1–1.6	0.6
S. pneumoniae	7	<0.1–0.6	0.1
S. faecalis	16	25–50	50
H. influenzae	9	0.2	0.2
N. gonorrhoeae	12	0.6–3.1	1.6
E. coli	59	0.2–200	0.8
K. pneumoniae	32	0.6–50	3.1
E. aerogenes	21	0.8–50	25
E. cloacae	11	12.5–>400	100
P. mirabilis	42	0.8–125	3.1
P. morganii	20	12.5–>400	100
P. vulgaris	15	1.6–200	100
P. rettgeri	7	1.6–25	25
C. freundii	34	0.1–>400	3.1
Providencia	28	50–>400	100
S. sonnei	32	0.1–>400	1.6
Salmonella	32	0.1–12.5	0.6
Serratia	26	6.2–>400	>400
Acinetobacter	13	3.1–>400	200
Pseudomonas	13	>400	>400
Bacteroides	29	25–>400	100

The activity of cefazaflur compared to that of cefoxitin, cefamandole, and cefuroxime against β-lactamase-producing, cephalothin-resistant isolates as is given in Table 3. Cefazaflur had inhibitory values similar to those of cefoxitin against *E. coli* and to all three agents against *Klebsiella.* Against the other organisms it was less active than the other three new agents. The activity of one of the new β-lactamase-resistant cephalosporins could not be used to predict the activity of cefazaflur.

Table 4 demonstrates the stability of cefazaflur to the β-lactamases of different bacteria. It is less stable than cefamandole against some type 1, induced β-lactamases, and it lacks the general β-lactamase stability of cefoxitin.

DISCUSSION

These studies confirm earlier data on the broad in vitro activity of cefazaflur against gram-positive cocci and many of the members of the *Enterobacteriaceae.* The activity of this agent against gram-positive species is similar to that which we have reported for cefamandole and cefuroxime (4, 6). In contrast to some of the earlier studies (1, 2, 4), we did not find this agent to be as active against *Enterobacter* species, *Serratia,* or indole-positive *Proteus,* such as *P. morganii* and *P. rettgeri,* as was cefamandole or cefoxitin. Cefazaflur is more stable to β-lactamase hydrolysis than cephalothin, but does not have the stability of cefoxitin.

Whether this agent would offer any advantages over cefamandole, cefuroxime, or cefoxitin is not apparent from the in vitro data, and animal protection studies comparing this agent to these new compounds are not available. Counts et al. (3) observed a marked inoculum and medium effect on this agent. Further studies

TABLE 2. *Comparative activity of cefazaflur and other β-lactamase agents*

Organism (no. of strains)	MIC (μg/ml) required for:[a]							
	50% of strains				90% of strains			
	CFZ	CET	CFM	CFX	CFZ	CET	CFM	CFX
S. aureus (20)	0.4	0.1	0.1	1.6	0.8	0.2	0.8	3.1
E. coli (25)	1.6	6.2	0.8	3.1	3.1	25	6.2	6.2
K. pneumoniae (35)	3.1	3.1	0.8	3.1	12.5	50	25	25
E. cloacae (20)	6.2	50	1.6	200	200	>400	12.5	>400
C. freundii (20)	6.2	50	1.6	100	>400	>400	6.2	>400
Salmonella (22)	0.2	6.3	0.8	1.6	6.2	50	12.5	3.1
Proteus, indole positive (20)	100	>400	12.5	6.2	>400	>400	100	12.5
Providencia (20)	100	>400	25	3.1	>400	>400	>400	6.2
Serratia (20)	200	>400	25	12.5	>400	>400	>400	>400
Bacteroides (20)	50	50	50	6.2	>400	>400	>400	25

[a] CFZ, Cefazaflur; CET, cephalothin; CFM, cefamandole; CFX, cefoxitin.

TABLE 3. *Activity of cefazaflur compared with that of other β-lactamase-stable penicillins against cephalothin-resistant isolates*

Organism	No. of isolates	MIC (μg/ml) of the following antibiotics:[a]				
		CET	CFZ	CFM	CFX	CFU
E. coli	1	100	12.5	100	12.5	12.5
E. coli	2	50	6.2	6.2	6.2	12.5
E. coli	3	50	6.2	25	6.2	50
E. coli	4	100	3.1	6.2	6.2	25
K. pneumoniae	1	200	6.2	200	1.6	3.1
K. pneumoniae	2	50	12.5	25	1.6	1.6
K. pneumoniae	3	100	1.6	25	3.1	3.1
E. cloacae	1	50	12.5	1.6	100	3.1
E. cloacae	2	>400	>400	400	100	200
E. cloacae	3	>400	>400	50	400	50
E. cloacae	4	100	>400	1.6	200	6.2
C. freundii	1	>400	>400	>400	>400	>400
C. freundii	4	100	100	0.4	200	1.6
P. vulgaris		400	200	6.2	12.5	25
P. morganii		400	200	3.1	12.5	6.2
P. morganii		400	400	6.2	100	100
P. rettgeri		400	1.6	3.1	12.5	3.1
P. rettgeri		400	25	3.1	3.1	3.1

[a] CET, Cephalothin; CFZ, cefazaflur; CFM, cefamandole; CFX, cefoxitin; CFU, cefuroxime.

TABLE 4. *β-Lactamase stability of cefazaflur compared with those of cefoxitin and cefamandole*[a]

Source of enzyme	Type of β-lactamase[b]	Relative rate of hydrolysis of:[c]		
		CFZ	CFM	CFX
Citrobacter	I	64	0	0
Enterobacter	I	0	0	0
Acinetobacter	I	100	91	0
Providencia	I	80	71	0
E. coli	III	10	20	0
Klebsiella	IV	100	100	0
Shigella	V	205	0	0

[a] β-Lactamase activity was determined by the spectrophotometric method, using change in absorbance at 255 nm. Reaction contained 0.4 mM substrate, and hydrolysis of cephalothin was set at 100.

[b] Richmond classification.

[c] CFZ, Cefazaflur; CFM, cefamandole; CFX, cefoxitin.

are needed to ascertain the role that this agent might play in the chemotherapy of infections.

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