IN VITRO STUDIES WITH CEFAZAFLUR AND OTHER PARENTERAL CEPHALOSPORINS

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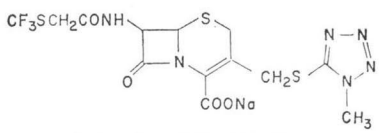
Cefazaflur has a broad-spectrum of *in vitro* antibacterial activity equal to or greater than that of the commercially-available cephalosporins. In addition, cefazaflur has activity against isolates of *Enterobacter*, *Citrobacter* and indole-positive *Proteus*; however, this activity decreased markedly when the MIC determinations were carried out with a large inoculum size. A similar inoculum effect was observed with cefamandole, however, cefoxitin's activity was relatively unchanged at increased inoculum sizes. Human serum had a relatively small effect on the *in vitro* activity of cefazaflur.

Cefazaflur, a new cephalosporin for parenteral administration is currently under clinical investigation. This cephalosporin has been reported to have potent and broad-spectrum *in vitro* and *in vivo* activity when compared with the commercially-available cephalosporins.[1,6] Its spectrum of activity is somewhat broader than the available cephalosporins.[1,2,5,11] It is highly stable to the activity of β-lactamases produced by staphylococci[7]. Cefazaflur has been reported to have good activity against anaerobic bacteria but was less active than cefoxitin against *Bacteroides fragilis*[10]. Cefazaflur is bound to serum proteins to about the same extent as is cephalothin, however, serum levels and half-life and urinary recovery, after parenteral administration, are somewhat greater than that of cephalothin[3,4,9]. Intramuscular administration of cefazaflur is less painful than that produced by cephalothin[3].



Cefazaflur (SK&F59962)

These studies were in part presented at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy and deal with the effect of human serum and inoculum size on the activity of cefazaflur.[2]

Materials and Methods

Approximately 500 isolates of bacteria were employed. The organisms were obtained as clinical isolates from various geographical locations in the United States and are part of the SK&F culture collection. The inoculum for each experiment was prepared from an appropriate dilution of a log phase culture grown in Trypticase Soy broth. All of the studies were carried out with a single batch of MUELLER-HINTON broth. The minimum inhibitory concentrations (MIC) were determined by a semi-automated Microtiter broth dilution technique (Cooke Engineering Co.). Serial two-fold dilutions of antibiotics were prepared in MUELLER-HINTON broth. The final inoculum size, unless otherwise stated, was approximately 10^5 organisms/ml test medium. For *Streptococcus pyogenes* and *Streptococcus pneumoniae*, TODD-HEWITT broth with an inoculum size of approximately 10^7 organisms/ml

was employed. The Microtiter plates (Cooke Engineering Co.) were incubated overnight in ambient air at 37°C. The MIC was defined as the lowest concentration of antibiotic in which there was no visible growth. MIC values were determined in separate experiments with and without human serum in the test medium. The inactivated, pooled batch of serum when added to the medium constituted 50% of the final test medium.

Cefazolin (SK&F), cephalothin (Lilly), cephapirin (Bristol), and cephaloridine (Lilly) were obtained as commercial preparations. Cefazaflur and cefamandole were prepared in our laboratory and the other experimental cephalosporins were kindly supplied as research samples (cefoxitin, Merck; cefuroxime, Glaxo).

Results and Discussion

Table 1 summarizes the minimum inhibitory concentrations (MIC) obtained with cefazaflur and commercially-available parenteral cephalosporins against 488 bacterial clinical isolates. Cefazaflur showed excellent activity against the gram-positive bacteria with the median MIC values all below 1 mcg/ml. Fig. 1 plots the cumulative percent of penicillin-sensitive and penicillin-resistant staphylococci inhibited by the four cephalosporins studied. All the cephalosporins showed comparable activity against the staphylococci except for cephaloridine which was more active than the others against penicillin-sensitive *Staphylococcus aureus*.

Fig. 1. *In vitro* activity of selected cephalosporins against 100 clinical isolates of *Staphylococcus aureus*

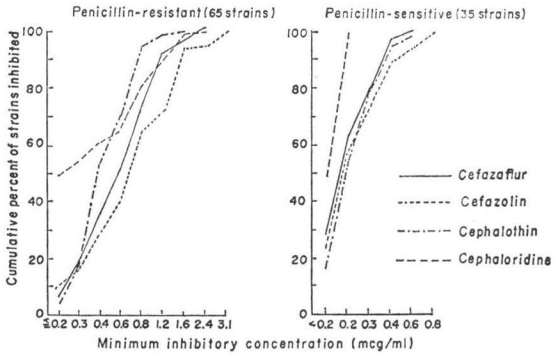


Table 1. Activity of cephalosporins against bacterial clinical isolates

Bacterial species	No. isolates	Median minimum inhibitory concentration (mcg/ml)			
		Cefazaflur	Cefazolin	Cephalothin	Cephaloridine
S. aureus (penicillin-sensitive)	35	0.3	0.3	0.2	0.2
S. aureus (penicillin-resistant)	65	0.6	0.8	0.4	0.3
Strep. pyogenes	35	0.16	0.16	0.16	0.02
Strep. pneumoniae	10	0.16	0.10	0.24	0.08
E. coli	79	0.4	1.6	6.3	3.1
K. pneumoniae	68	0.6	1.6	3.1	4.7
P. mirabilis	48	1.2	3.1	4.7	6.3
Salmonella sp.	24	0.4	1.6	3.1	3.1
Ent. aerogenes	11	1.6	3.1	25	75
Ent. cloacae	49	6.3	200	200	200
Enterobacter sp.*	10	9.4	>200	200	>200
Citrobacter	10	28	250	63	220
Proteus rettgeri	10	125	500	500	500
Proteus vulgaris	10	187	250	250	187
Proteus morganii	10	500	250	>500	500
Herellea	4	312	375	312	156
Providencia	10	375	>500	500	500

* Seven isolates of *E. liquefaciens*, 2 *E. agglomerans*, 1 *E. hafniae*.

Fig. 2. *In vitro* activity of selected cephalosporins against 79 clinical isolates of *Escherichia coli*

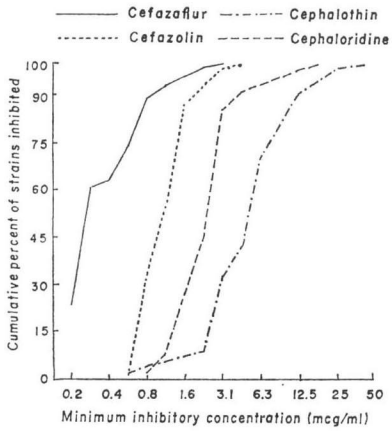


Fig. 3. *In vitro* activity of selected cephalosporins against 68 clinical isolates of *Klebsiella pneumoniae*

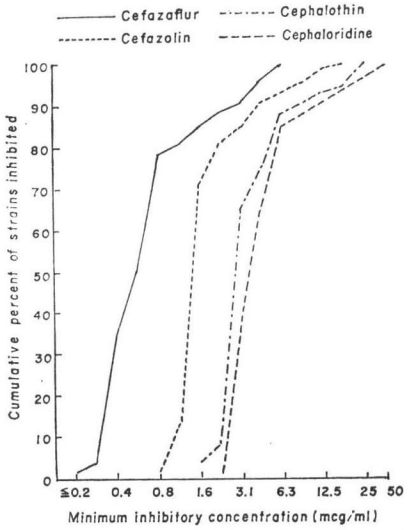


Fig. 4. *In vitro* activity of selected cephalosporins against 48 clinical isolates of *Proteus mirabilis*

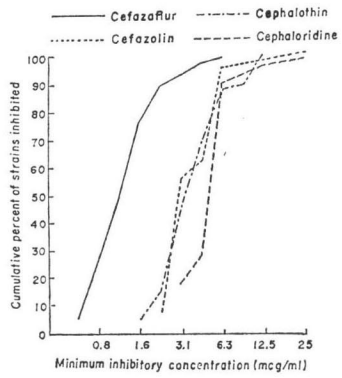


Fig. 5. *In vitro* activity of selected cephalosporins against 49 clinical isolates of *Enterobacter cloacae*

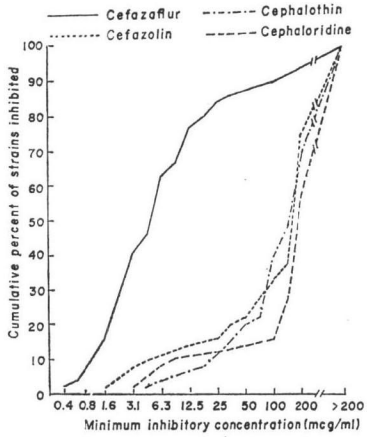


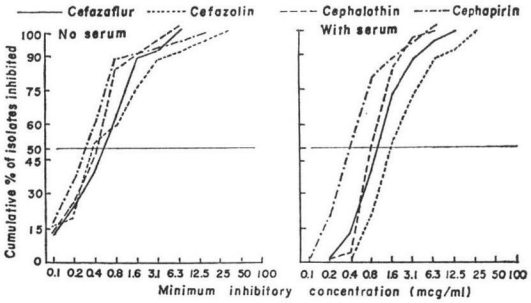
Table 2. Effect of human serum on activity of selected cephalosporins

Bacteria	No. strains	Median MIC values (mcg/ml)							
		Cefazaflur		Cefazolin		Cephalothin		Cephapirin	
		Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum
S. aureus	25	0.8	1.6	0.4	1.6	0.8	1.6	0.4	0.4
E. coli	25	0.4	0.8	1.6	6.3	6.3	25	12.5	25
P. mirabilis	22	2.4	3.2	4.7	25	3.2	6.3	6.3	6.3
K. pneumoniae	25	0.8	1.6	1.6	6.3	3.2	12.5	3.2	6.3
E. aerogenes	19	1.6	6.3	6.3	25	25	100	25	100

* MUELLER-HINTON Broth with $\sim 1 \times 10^5$ CFU/ml test medium.

Cefazaflur activity against the most common gram-negative bacteria also was of high order. Cefazaflur was clearly more active than cefazolin, cephalothin and cephaloridine against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Figs. 2, 3, 4). In addition, cefazaflur was active against *Enterobacter* species whereas cephalothin and cephaloridine were inactive against these bacteria (Fig. 5) and cefazolin was active only against *Enterobacter aerogenes* (Table 1). Against indole-positive *Proteus, Citrobacter, Herellea* and *Providencia* none of the cephalosporins tested showed significant activity, however, cefazaflur was active against some of the strains tested. These data are in general agreement with the recent report of VERBIST, although he reported a higher percentage of the indole-positive *Proteus* and *Providencia* strains to be susceptible to cefazaflur.[11]

Fig. 6. Effect of human serum on the *in vitro* activity of selected cephalosporins against 25 clinical isolates of *Staphylococcus aureus*



A series of studies which are summarized in Table 2, were then carried out to examine the effect of human serum on the activity of cefazaflur and control cephalosporins. Fig. 6 shows the activity

Fig. 7. Effect of human serum on the *in vitro* activity of selected cephalosporins against 25 clinical isolates of *Escherichia coli*

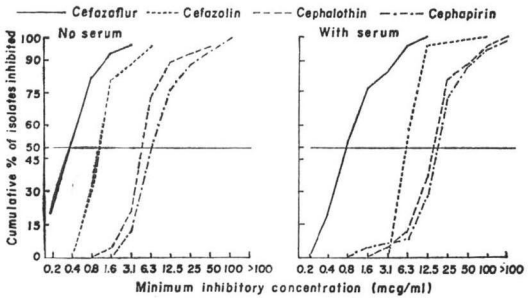


Fig. 8. Effect of human serum on the *in vitro* activity of selected cephalosporins against 25 clinical isolates of *Klebsiella pneumoniae*

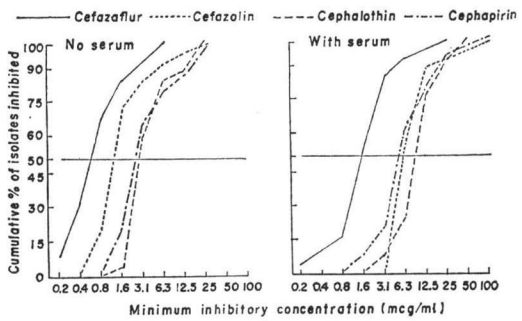


Fig. 9. Effect of human serum on the *in vitro* activity of selected cephalosporins against 22 clinical isolates of *Proteus mirabilis*

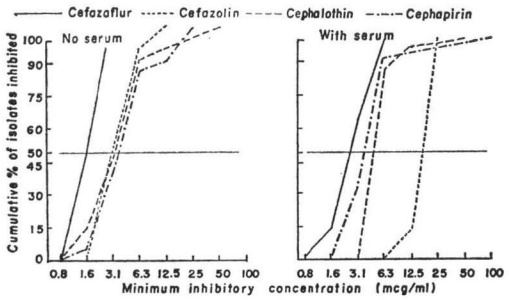


Fig. 10. Effect of human serum on the *in vitro* activity of selected cephalosporins against 19 clinical isolates of *Enterobacter aerogenes*

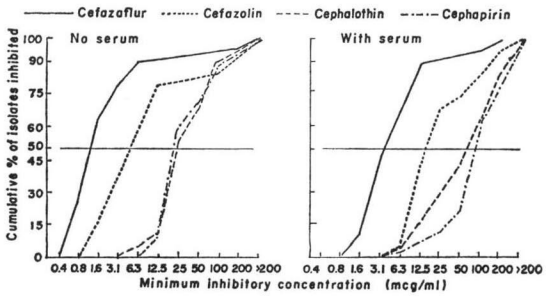


Table 3. Effect of inoculum size on *in vitro* activity of selected cephalosporins

Cephalosporin	Median minimum inhibitory concentration (mcg/ml)								
	Staph. aureus			*E. coli*			*Klebsiella*		
	10^3	10^5	10^7	10^3	10^5	10^7	10^3	10^5	10^7
Cefazaflur	0.4	0.8	3.1	0.4	0.4	6.3	0.2	0.4	1.2
Cefamandole	0.8	1.6	6.3	0.2	0.4	1.6	0.6	0.8	3.1
Cephalothin	0.4	0.4	1.6	3.1	6.3	50	1.6	2.4	12.5
Cefazolin	0.8	1.6	6.3	1.6	1.6	3.1	0.8	1.6	3.1
Cefoxitin	3.1	3.1	3.1	3.1	3.1	6.3	2.4	3.1	4.7
Cefuroxime	1.6	1.6	1.6	3.1	3.1	6.3	1.2	1.6	3.1
Cephapirin	0.4	0.4	3.1	3.1	6.3	100	1.2	1.6	12.5

Table 4. Effect of inoculum size on *in vitro* activity of selected cephalosporins

Cephalosporin	Median minimum inhibitory concentration (mcg/ml)							
	Citrobacter		*Proteus* (indole-positive)			*Enterobacter* sp.		
	10^5	10^7	10^5	10^7	10^9	10^4	10^6	10^8
Cefazaflur	1	250	8	125	500	4	8	500
Cefamandole	1	63	$\leqq 0.5$	1	125	4	4	63
Cefoxitin	16	32	2	2	8	63	63	125
Cefuroxime	4	32	4	8	125	4	8	31

of these cephalosporins against 25 clinical isolates of *S. aureus* with and without 50% human serum in the medium. With the exception of cephapirin which showed only a slight loss in activity, all of the cephalosporins showed a 2~4-fold loss in activity in the presence of serum. A similar experiment employing 25 *E. coli* strains is shown in Fig. 7. Again, the activity of all of the cephalosporins was reduced in serum, however, the median MIC value for cefazaflur in serum (0.8 mcg/ml) was clearly superior to cefazolin (6.3 mcg/ml), cephalothin (25 mcg/ml) and cephapirin (25 mcg/ml). Against *P. mirabilis* and *K. pneumoniae* isolates, cefazolin and cephalothin showed a 4-fold loss in median MIC values whereas cefazaflur and cephapirin showed little or no change in activity (Figs. 8, 9). Of the four cephalosporins examined for serum effects against *E. aerogenes*, only cefazaflur and cefazolin showed significant activity (Fig. 10). Cefazaflur was clearly more active than cefazolin against *E. aerogenes* in both broth and serum. It is of interest to note that although cephalothin is less bound to serum proteins than is cefazolin (65% *vs.* 85%), its activity in the presence of serum was poorer than that of cefazolin. Cephalothin has been reported to be degraded *in vitro* by human serum after incubation at 37°C[8].

The effect of inoculum size on *in vitro* activity of cefazaflur and control cephalosporins was next examined. In these studies, 10 organisms each of *S. aureus, E. coli, K. pneumoniae*, indole-positive *Proteus*, *Enterobacter* species and *Citrobacter* at various inoculum sizes were tested against seven parenteral cephalosporins. Table 3 shows the results obtained with *S. aureus, E. coli* and *K. pneumoniae* isolates. Cefazaflur, cefamandole, cephalothin and cefazolin all experienced a 4-fold increase in the median MIC values against *S. aureus* when the inoculum size was increased from 10^5 to 10^7 organisms/ml. Cephapirin showed an 8-fold loss in activity but no significant inoculum effect was observed with cefoxitin or cefuroxime. Against *E. coli*, cefazaflur showed a significant activity loss at the high inoculum size and 6.3 mcg/ml were required to inhibit 50% of the isolates. The median MIC for cephalothin (50

mcg/ml) and cephapirin (100 mcg/ml) at this high inoculum size also had increased significantly. Against *Klebsiella* isolates, there was less of an inoculum effect; however, the MIC values for cephalothin and cephapirin at an inoculum size of 10^7 organisms/ml was relatively poor (12.5 mcg/ml).

A more dramatic effect of inoculum size was observed with organisms that tend to show a variable response to the newer cephalosporins (Table 4). Against these bacterial isolates (*Citrobacter*, indole-positive *Proteus* and *Enterobacter* sp.), cefazaflur showed good median MIC values at inoculum sizes ranging for 10^4 to 10^6 organisms/ml. At an inoculum level of 10^7 organisms/ml, cefazaflur showed poor activity against strains of indole-positive *Proteus* and similar poor activity at 10^8 organisms/ml against *Enterobacter* species. Cefamandole showed a similar inoculum response but tended to be more active than cefazaflur against these organisms. Cefoxitin shows very little change in activity with increased inoculum size but was generally poor against the *Enterobacter* species. Cefuroxime was intermediate in response between cefoxitin and cephamandole, but, some break in activity was observed at the higher inoculum size especially against isolates of indole-positive *Proteus*. COUNTS recently reported a similar high inoculum effect with indole-positive *Proteus* and *Enterobacter* species[5].

Acknowledgements

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