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BL-S786 (Ceforanide), a New Parenteral Cephalosporin: In Vitro Studies

SUSANNE S. WEAVER, BARBARA M. LEBLANC, AND GERALD P. BODEY

Department of Developmental Therapeutics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

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BL-S786 (ceforanide) is a new cephalosporin which showed broad-spectrum activity in vitro against 453 clinical isolates. At a concentration of 3.12 μg/ml, it inhibited greater than 75% of isolates of *Escherichia coli, Klebsiella* spp., *Proteus mirabilis, Staphylococcus aureus, Streptococcus pyogenes,* and *Streptococcus pneumoniae.* Essentially no activity was observed against isolates of *Serratia marcescens,* and only minimal activity was observed against *Enterobacter* spp. Its activity was directly related to the size of the inoculum. The minimal bactericidal concentrations were similar to the minimal inhibitory concentrations for isolates of all organisms except *S. aureus* and *S. pyogenes.* The minimal bactericidal concentrations were considerably higher than the minimal inhibitory concentrations for these organisms.

BL-S786 (ceforanide), 7-{α-(2-aminomethylphenyl) acetamido}-3-{(1-carboxy-methyltetrazol-5-ylthio) methyl}-3-cephem-4-carboxylic acid, is a new semisynthetic, parenteral cephalosporin which has been shown to have a broader spectrum of activity in vitro than currently available cephalosporins and comparable activity to investigational drugs such as cefamandole and cefoxitin (1, 3-5). Its activity against major pathogens, which are a cause of morbidity and mortality among hospitalized patients, warranted further study to confirm these original observations. Also, additional comparative data with cefamandole, cephalexin, cefoxitin, cephalothin, and cephapirin were obtained.

Susceptibility tests were performed on 305 clinical isolates of gram-negative bacilli and 148 clinical isolates of gram-positive cocci by a microserial dilution technique. Organisms were tested in triplicate simultaneously and included 100 isolates of *Escherichia coli,* 100 isolates of *Klebsiella* spp., 53 isolates of *Proteus mirabilis,* 25 isolates of *Enterobacter* spp., 27 isolates of *Serratia marcescens,* 50 isolates of penicillin G-resistant *Staphylococcus aureus,* 40 isolates of penicillin G-susceptible *S. aureus,* 43 isolates of *Streptococcus pyogenes,* and 15 isolates of *Streptococcus pneumoniae.* Isolates of *S. aureus* resistant to 25 μg or more of penicillin G per ml were selected as penicillin G-resistant, and those isolates inhibited by 0.10 μg or less per ml were selected as penicillin G-susceptible, as determined by a broth dilution method. All gram-negative bacilli were cultured from blood specimens obtained from cancer patients at this institution. Gram-positive cocci were cultured from various sites from hospitalized patients, some of whom did not have cancer.

All isolates of gram-negative bacilli and of *S. aureus* were incubated in Mueller-Hinton broth at 37°C for 18 h. Isolates of *S. pyogenes* and *S. pneumoniae* were incubated in tryptose-phosphate broth in a 37°C CO_2 incubator for 18 h. Overnight cultures of gram-negative bacilli and *S. aureus* isolates were diluted to an estimated 10^5 cells per ml, and isolates of *S. pyogenes* and *S. pneumoniae* were diluted to 10^6 cells per ml. The actual concentrations of of organisms were confirmed by plate counting. A 0.05-ml sample was used as the inoculum and was added manually to twofold serial dilutions of the antibiotics made automatically on a Canalco Autotiter IV (Canalco Autotiter IV instruction manual). Mueller-Hinton broth was used for testing all organisms except *S. pyogenes* and *S. pneumoniae,* for which tryptose-phosphate broth was used. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of drug which suppressed visible growth after incubation at 37°C for 18 h for gram-negative bacilli and isolates of *S. aureus* and after incubation at 37°C in a CO_2 incubator for 24 h for isolates of *S. pyogenes* and *S. pneumoniae.* The minimal bactericidal concentration (MBC) was defined as the lowest concentration of drug which yielded less than five colonies on subculture on sheep blood agar (99% kill) and incubation at 37°C for 18 h. *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and an isolate of *P. mirabilis* were included as control organisms in all tests.

BL-S786 and cephapirin were supplied by Bristol Laboratories, Syracuse, N.Y.; cefamandole, cephalothin, and cephalexin were supplied by Eli Lilly and Co., Indianapolis, Ind.; and cefoxitin was supplied by Merck, Sharp and Dohme, Rahway, N.J.

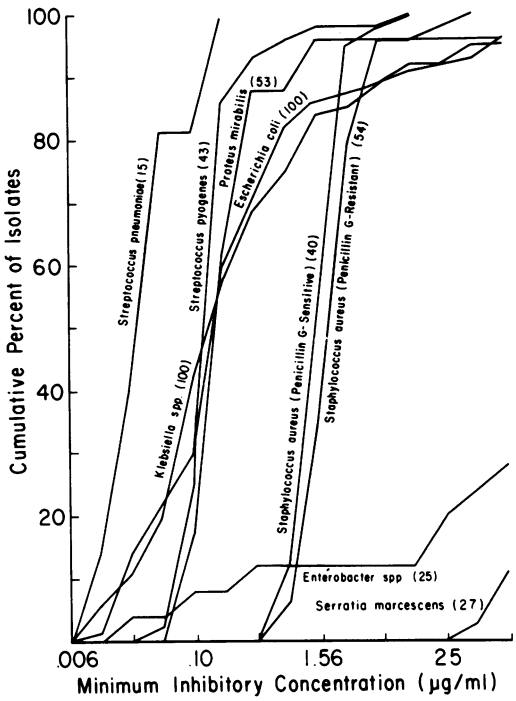

FIG. 1. *In vitro activity of BL-S786 against gram-positive coci and gram-negative bacilli.*

The in vitro activity of BL-S786 against gram-positive cocci and gram-negative bacilli is shown in Fig. 1. All 15 of the isolates of *S. pneumoniae* and 86% of the isolates of *S. pyogenes* were inhibited by this antibiotic at a concentration of 0.20 µg/ml. It inhibited 95% of isolates of penicillin G-susceptible *S. aureus* and 80% of isolates of penicillin G-resistant *S. aureus* at a concentration of 3.12 µg/ml. It was active against *Klebsiella* spp., *P. mirabilis,* and *E. coli,* inhibiting 84, 96, and 86% of the isolates, respectively, at a concentration of 1.56 µg/ml. It showed essentially no activity against isolates of *S. marcescens.* BL-S786 inhibited only 20% of isolates of *Enterobacter* spp. at a concentration of 25 µg/ml.

The effect of inoculum size on the MICs and MBCs of BL-S786 for 10 isolates each of *K. pneumoniae, E. coli,* and *P. mirabilis* was determined (Fig. 2). An increase in the inoculum concentration from 10^5 to 10^7 cells per ml significantly reduced the activity of this drug.

TABLE 1. *Comparison of MIC and MBC of BL-S786 against isolates of S. aureus* and *S. pyogenes*

Organism	Total no. of isolates	No. of isolates at MBC/MIC ratio:					
		1	2	4	8	16	>16
S. aureus (penicillin G-susceptible)	40	22	8	0	0	1	9
S. aureus (penicillin G-resistant)	50	2	7	1	1	4	35
S. pyogenes	43	22	3	0	3	0	15

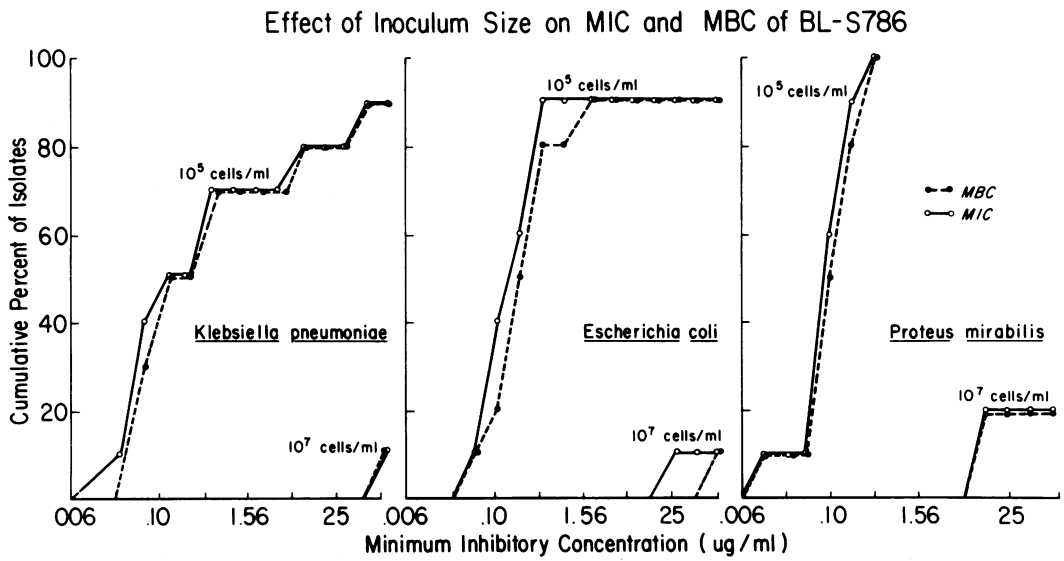

FIG. 2. *Effect of inoculum size on MIC and MBC of BL-S786.*

TABLE 2. *Comparative in vitro activity of BL-S786 and five other cephalosporins against 323 clinical isolates*

Organism (No. of isolates)	Drug[a]	Cumulative % inhibited at MIC (μg/ml):				
		0.20	0.78	3.12	12.5	25
E. coli (50)	786	61	82	87	92	92
	CEF	58	76	88	92	93
	CLX		2	52	88	91
	CFN	2	25	79	98	99
	CLN		2	21	70	83
	CPN			11	60	75
K. pneumoniae (50)	786	58	75	85	91	92
	CEF	14	50	75	83	86
	CLX		3	54	92	94
	CFN		21	66	90	95
	CLN	1	9	46	74	84
	CPN	1	8	45	70	82
P. mirabilis (50)	786	62	96			
	CEF	21	80	93	96	98
	CLX			2	81	93
	CFN		2	91	98	100
	CLN		4	57	94	96
	CPN		2	53	94	96
Enterobacter spp. (25)	786					20
	CEF		12	28	40	52
	CLX				12	
	CFN				8	
	CLN				4	
	CPN				4	
S. aureus (penicillin G-susceptible) (40)	786		10	95	100	
	CEF	90	100			
	CLX		10	98		
	CFN		3	88	100	
	CLN	100				
	CPN	100				
S. aureus (penicillin G-resistant) (50)	786		6	80		98
	CEF	24	98	98	100	
	CLX		4	42	92	96
	CFN			98	98	100
	CLN	84	98		100	
	CPN	86	98	98	100	
S. pyogenes (43)	786	86	96	98	100	100
	CEF	98	100			
	CLX	86	91	98	98	
	CFN	41	98	100		
	CLN	98	100			
	CPN	100				
S. pneumoniae (15)	786	100				
	CEF	100				
	CLX		26	100		
	CFN	20	100			
	CLN	100				
	CPN	100				

[a] 786, BL-S786; CEF, cefamandole; CLX, cephalexin; CFN, cefoxitin; CLN, cephalothin; CPN, cephapirin.

These same isolates were used to determine the activity of BL-S786 in different media. This antibiotic was slightly more active in Trypticase soy broth against isolates of *K. pneumoniae,* but its activity against isolates of *P. mirabilis* and *E. coli* was not substantially different in the various media tested.

The MICs and the MBCs were similar for isolates of all organisms except *S. aureus* and *S. pyogenes.* For 9 isolates of penicillin G-susceptible *S. aureus,* 35 isolates of penicillin G-resistant *S. aureus,* and 14 isolates of *S. pyogenes* the MBCs were more than 16 times the MIC (Table 1).

The activity of BL-S786 was compared with that of cefamandole, cephalexin, cefoxitin, cephalothin, and cephapirin by using 50 isolates each of *E. coli, Klebsiella* spp., *P. mirabilis,* and penicillin G-resistant *S. aureus*; 25 isolates of *Enterobacter* spp.; 40 of penicillin G-susceptible *S. aureus;* 43 of *S. pyogenes;* and 15 of *S. pneumoniae* (Table 2). BL-S786 was comparable in activity to cefamandole against isolates of *E. coli,* inhibiting 87% at a concentration of 3.12 μg/ml, whereas cefamandole inhibited 88% and cefoxitin inhibited 79%. It was the most active antibiotic against isolates of *Klebsiella* spp. and *P. mirabilis.* At a concentration of 0.78 μg/ml, it inhibited 75% of the isolates of *Klebsiella* spp. and 96% of those of *P. mirabilis,* whereas cefamandole inhibited 50 and 80%, respectively. Cefoxitin, the next most active antibiotic inhibited only 21 and 2%, respectively. BL-S786 and cefamandole were the only antibiotics which exhibited any activity against isolates of *Enterobacter* spp. at concentrations lower than 6.25 μg/ml. Cefamandole was considerably more active at higher concentrations, however, inhibiting 52% at a concentration of 25 μg/ml, whereas BL-S786 inhibited only 20%. Cephalexin and cefoxitin inhibited only 12 and 8%, respectively, at this same concentration.

BL-S786 was not as active against isolates of gram-positive cocci as cephalothin, cephapirin, or cefamandole at concentrations less than 0.50 μg/ml. It was comparable to cefoxitin and cephalexin in activity against isolates of penicillin G-susceptible *S. aureus* and *S. pyogenes,* inhibiting 95 and 98% of isolates, respectively, at a concentration of 3.12 μg/ml. It was slightly less active than cefoxitin and more active than cephalexin against isolates of penicillin G-resistant *S. aureus,* inhibiting 80% of isolates at a concentration of 3.12 μg/ml, whereas cefoxitin inhibited 98% and cephalexin inhibited 42%. It was considerably more active than cefoxitin or cephalexin against isolates of *S. pneumoniae,* inhibiting 100% of isolates at a concentration of 0.20 μg/ml, whereas cefoxitin inhibited only 20%. Cephalexin exhibited no activity at this concentration.

BL-S786 exhibited broad-spectrum activity in vitro against gram-positive cocci and gram-negative bacilli. It was the most active cephalosporin against isolates of *Klebsiella* spp. and *P. mirabilis* and was as active as cefamandole against isolates of *E. coli.* Earlier studies compared its activity to that of cephalothin (1, 3–5), cefamandole, and cefoxitin (1, 4, 6). In general, our results were similar, but Shadomy et al. found BL-S786 to be more active than cefamandole against isolates of *E. coli* (6). In our study, BL-S786 was not as active against isolates of *Enterobacter* spp. as reported elsewhere (3–6), but the activity of cefamandole, cefoxitin, and cephalothin against these isolates compared well with earlier studies in our laboratory (2), suggesting that our group of isolates is generally more resistant. Jones et al. found that the inhibitory activity of BL-S786 and cefamandole against *Enterobacter* spp. was markedly reduced when bactericidal values were considered (4). Earlier studies in our laboratory showed little variation between MICs and MBCs for *Enterobacter* spp. (2), indicating that our isolates are inhibited at concentrations more nearly bactericidal. Although other antibiotics showed greater activity against isolates of gram-positive cocci, BL-S786 was active against these at concentrations which should be achieved in humans with standard parenteral dosage. Considering the differences in MICs and MBCs for isolates of *S. aureus* and *S. pyogenes,* however, this antibiotic may not prove as effective in vivo against infections caused by these organisms. Aswapokee et al. found that the difference between inhibitory and bactericidal concentrations appeared to be related to β-lactamase destruction of the antibiotic (1).

In vivo studies have shown that BL-S786 reached higher peak serum concentrations, that its concentration declined at a slower rate than other cephalosporins, and that it was the most efficacious drug in treatment of infections in mice (5, 6).

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