BL-S786, A NEW PARENTERAL CEPHALOSPORIN. II

IN VITRO ANTIMICROBIAL ACTIVITY COMPARISON WITH SIX RELATED CEPHALOSPORINS

R. N. JONES

Clinical Microbiology Division, Kaiser Foundation Hospital Laboratories
Portland, Oregon 97217, U.S.A.

C. THORNSBERRY

Antimicrobic Testing Laboratory, Center for Disease Control
Atlanta, Georgia 30333, U.S.A.

A. L. BARRY

Clinical Microbiology Laboratories, University of California (Davis)
Sacramento Medical Center, Sacramento, California 95817, U.S.A.

P. C. FUCHS

Department of Pathology, St. Vincent Hospital and
Medical Center, Portland, Oregon 97225, U.S.A.

T. L. GAVAN

Department of Microbiology, The Cleveland Clinic
Cleveland, Ohio 44106, U.S.A.

and E. H. GERLACH

Microbiology Laboratory, St. Francis Hospital
Wichita, Kansas 67214, U.S.A.

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BL-S786 was compared by *in vitro* studies with 6 other parenteral cephalosporins (cefamandole, cefazolin, cefoxitin, cephaloridine, cephalothin and cephradine). The following parameters were assessed: Comparative MICs against a wide variety of bacterial isolates, MIC/MBC comparisons and the effect of inoculum size on the MIC. BL-S786 showed the greatest antimicrobial activity against *K. pneumoniae*, *C. diversus* and *Salmonella* species; was equal to cefamandole against *E. coli*, *E. agglomerans* and *P. mirabilis*; and was second to cefamandole against *Shigella*, *E. tarda*, *C. freundii*, *E. cloacae*, *E. aerogenes* and the pathogenic Neisseriae. Essentially no activity against *Serratia* and *Pseudomonas* species was observed. Compared to the other cephalosporins tested, BL-S786 showed poor activity against staphylococci and streptococci. For most species tested, the MBC of the various cephalosporins was the same or within one dilution of their respective MICs. However, for *Enterobacter* and indole-positive *Proteus* species, the MBC of BL-S786 and cefamandole was usually $\geq$8-fold higher than the MICs. Cefoxitin, on the other hand, showed little MIC/MBC variations against indole-positive *Proteus* species. Inoculum size had only a small effect on the MICs against most gram-negative species—in some instances > 64-fold increases in MIC resulted by increasing inoculum size from 10^5 to 10^7 organisms per ml.

BL-S786 is a new parenterally administered semisynthetic cephalosporin having a broader spectrum of antimicrobial activity as compared with the currently available cephalosporins[1]. In addition, favorable pharmacokinetics and infection studies have been reported in humans and in experimental animals respectively[2]. This *in vitro* study directly compares the antimicrobial characteristics of BL-

S786 with that of currently available cefazolin, cephaloridine, cephalothin and cephradine, plus two additional new promising investigational cephalosporins, cefamandole and cefoxitin.

Materials and Methods

Antibiotics:

The cephalosporin laboratory standard powders were supplied by the following pharmaceutical companies: BL-S786 from Bristol Laboratories, Syracuse, New York; cefamandole, cephaloridine, and cephalothin from Eli Lilly & Company, Indianapolis, Indiana; cefoxitin from Merck Sharp & Dohme, Rahway, New Jersey; Cephradine from E. R. Squibb & Sons, Princeton, New Jersey; and cefazolin from Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

Organisms:

A total of 407 bacterial isolates were provided by the collaborating laboratories for this study. This include 173 strains of the *Enterobacteriaceae*, 65 strains of non-enterobacteriaceae gram-negative bacilli, 117 strains of gram-positive cocci, and 52 strains of *Neisseria* species. They were further broken down into the following genus and species groups: 26 *E. coli*, 25 *Klebsiella pneumoniae*, 25 *Proteus mirabilis*, 6 *Citrobacter diversus*, 6 *Citrobacter freundii*, 5 *Edwardsiella tarda*, 6 *Salmonella* species, 17 *Serratia* species, 8 *Shigella* species, 24 *Enterobacter* species, 25 indole-positive *Proteus* species, 30 *Pseudomonas* species, 6 *Aeromonas hydrophilia*, 29 *Haemophilus influenzae* (10 β-lactamase producers), 35 *Staphylococcus aureus* (10 methicillin resistant), 18 *Staphylococcus epidermidis*, 23 *Streptococcus pneumoniae*, 22 *Streptococcus pyogenes*, 11 *Streptococcus faecalis*, 25 *Neisseria gonorrhoeae*, and 27 *Neisseria meningitidis*.

Multiple isolates were tested in duplicate by two of the collaborating laboratories (Center for Disease Control and the Sacramento Medical Center) in a manner previously reported[3]. No significant variation in results were encountered between the participating laboratories.

Antimicrobic Susceptibility Testing:

Minimum inhibitory concentrations (MICs) were determined by the microdilution broth method. MUELLER-HINTON broth was commercially dispensed in a single lot of plastic trays (Micro Media Systems, Campbell, California) and distributed to the testing laboratories. The trays were stored at $-60°C$ until inoculated. Prior to use the trays were thawed at room temperature (approximately $20 \sim 30$ minutes) and inoculated with disposable inoculators delivering 5 μl to each well.

The two laboratories differed only slightly in the method used to standardize the inoculum density. At the Center for Disease Control, a logarithmic phase broth culture was diluted to match the turbidity of a 0.5 MACFARLAND standard. The suspension was then diluted 1: 50 in sterile water containing 0.02% Tween 80 and dispensed as described earlier. Final inoculum achieved was 1×10^5 colony forming units (CFU) per ml. At the Sacramento Medical Center (SMC) the test organisms were inoculated into a small volume (0.5 ml) of brain-heart infusion broth and incubated $5 \sim 6$ hours at 35°C. This culture was then dilution 1: 100 in water (containing 0.02% Tween 80) and inoculated into trays, inoculum concentration of 5×10^5 CFU/ml.

The MIC was recorded as the lowest concentration totally inhibiting bacterial growth (clear well), after approximately 18 hours of incubation at 35°C in a forced air incubator. Occasionally, visible growth occurred in concentrations $1 \sim 2$ wells above the MIC, (the skipped-tube phenomenon).

For the testing of the fastidious streptococci including *S. pyogenes* and *S. pneumoniae*, the inoculum was standardized in MUELLER-HINTON broth containing 5% lysed rabbit blood and 0.1 ml of this adjusted cell suspension was added to each microdilution well, giving a final concentration of 1×10^5 CFU/ml. The MICs for *Haemophilus influenzae* were determined by suspending colonies directly in MUELLER-HINTON broth supplemented with 10% peptic digest of horse cells and 2% IsoVitaLex (BBL), adjusting to match a MACFARLAND 0.5 turbidity standard. This was further diluted to a concentration of 10^4 CFU/ml and 0.1 ml added to each well. Trays were incubated under increased CO_2 tension for both the streptococci and *Haemophilus influenzae*.

N. gonorrhoeae and *N. meningitidis* were tested by the agar dilution method. Proteose peptone

agar with 1% hemoglobin and 1% Kellogg's supplement, was prepared incorporating appropriate antibiotic concentrations. The inoculum was made by suspending colonies in MUELLER-HINTON broth, diluted to a concentration of 1×10^6 CFU/ml. Plates were then inoculated by a STEERS' replicator[4]. The MICs were determined after 24 hours of incubation in 5% CO_2 at 35°C.

Minimum bactericidal concentrations were determined for 76 organisms from five genera by subculturing 5 μl from each microtiter well. The 5 μl subcultures were transferred to MUELLER-HINTON agar (SMC) and to trypticase soy agar with 4% rabbit blood (CDC). The subcultures were made with multiple inoculum replicator onto a 150-mm Petri plate. After 48 hours of incubation, the endpoints were read as the lowest concentration yielding no more than 0.1% survivors (99.9% kill).

The effect of varying the inoculum concentrations on MIC endpoints was studied on 103 rapid growing facultative anaerobes. Trays were inoculated to achieve final concentrations of 10^3, 10^5, and 10^7 CFU/ml. MICs were interpreted as described above.

Results

MIC Comparisons

Table 1 summarizes the cumulative percentage susceptibility results for the *Enterobacteriaceae*, *Aeromonas hydrophila*, and *Pseudomonas* species to increasing concentrations of BL-S786 and six other parenteral cephalosporins. BL-S786 was clearly the most active cephalosporin against *Klebsiella pneumoniae*, *Citrobacter diversus* and *Salmonella* species by a two-fold dilution step. Comparable

Table 1. Cumulative percentage susceptibility of 11 gram-negative species (160 organisms) to increasing concentrations of BL-S786 and 6 other parenteral cephalosporins.

Organism (#)	Cephalosporin	Cumulative % inhibited at MIC (μg/ml) of									
		≤0.06	0.125	0.25	0.5	1	2	4	8	16	32
E. coli (26)	786	2	4	28	67	83	87	91			93
	CMD		9	30	78	83	85	89	98	100	
	CZ				9	70	85	87	89	93	96
	COX					11	48	80	87	93	100
	CLD					11	59	83	85	89	93
	CF						11	37	74	85	89
	CD							11	76	87	91
Klebsiella pneumoniae (25)	786	2		51	78	87	89	93	96	100	
	CMD			2	31	73	78	80	93	98	
	CZ				2	62	80	91	96	98	100
	COX				2		38	82	89	96	
	CLD				2		20	78	89	93	
	CF				2	4	44	78	87	93	96
	CD							22	89	93	96
Proteus mirabilis (25)	786			12	93	95	100				
	CMD			12	88	100					
	CZ					2	23	84	100		
	COX					5	33	81	91	100	
	CLD							42	88	100	
	CF					5	44	91	100		
	CD							2	9	77	93
Citrobacter diversus (6)	786			67		83	100				
	CMD				67				83	100	
	CZ					33	67	83		100	
	COX						50	83			100
	CLD						33	67	83		100
	CF						50	67	83	100	
	CD								83		100

(to be continued)

Table 1. (continued)

Organism (#)	Cephalosporin	Cumulative % inhibited at MIC (μg/ml) of									
		≤0.06	0.125	0.25	0.5	1	2	4	8	16	32
Citrobacter freundii (6)	786						33	50	83		
	CMD				33	67	83				
	CZ										
	COX						33		50		
	CLD								17		33
	CF							17		33	
	CD								50		67
Edwardsiella tarda (5)	786	20	60	100							
	CMD	60	100								
	CZ				40	100					
	COX				80	100					
	CLD					100					
	CF				20	60	100				
	CD						20	100			
Salmonella species (6)	786		17	83							
	CMD			17	83						
	CZ					83					
	COX					17	100				
	CLD						83				
	CF					17	83				
	CD							67	100		
Serratia species (17)[a]	786										
	CMD									23	77
	CZ										
	COX								27	77	95
	CLD										9
	CF										
	CD										
Shigella dysenteriae (8)	786				50	100					
	CMD		100								
	CZ					100					
	COX					100					
	CLD						100				
	CF							100			
	CD							100			
Aeromonas hydrophila (6)	786							17	33		56
	CMD			8	50	67	83	92		100	
	CZ							25		50	67
	COX				17		25	33	58	83	92
	CLD							17	33		42
	CF					8	17			25	58
	CD							42	58		
Pseudomonas species (30)[b]	786										
	CMD										7
	CZ										
	COX									3	17
	CLD										
	CF										
	CD										3

a. Includes *S. marcescens* (16) and *S. rubidea* (1).
b. Includes ten strains of *Ps. aeruginosa*, *Ps. cepacia* and *Ps. maltophilia*.
c. 786=BL-S786; CMD=cefamandole; CZ=cefazolin; COX=cefoxitin; CLD=cephaloridine; CF=cephalothin; CD=cephradine.

results were obtained with both cefamandole and BL-S786 for *E. coli* and *Proteus mirabilis* isolates. Cefamandole proved to be superior to BL-S786 against *Shigella* species, *Edwardsiella tarda*, and *Citrobacter freundii*. However, BL-S786 was more active than the other five cephalosporins. Only cefoxitin

Table 2. Minimal inhibitory concentration of BL-S786, cefamandole and cefoxitin against *Enterobacter* species and indole-positive *Proteus* species.

Organism (#)	BL-S786		Cefamandole		Cefoxitin	
	Range	Median	Range	Median	Range	Median
Enterobacter aerogenes (10)	0.5~>32[a]	4	0.5~>32	1	>32	>32
Enterobacter cloacae (10)	4~>32	16	1~>32	4	2~>32	>32
Enterobacter agglomerans (4)	0.25~2	0.5	0.125~2	0.5	2~8	4
Proteus morganii (11)	32~>32	>32	1~>32	8	2~>32	8
Proteus rettgeri (8)	0.06~>32	1	0.06~>32	1	1~>32	2
Proteus vulgaris (6)	4~>32	>32	16~>32	>32	2~>32	4

a. Minimal inhibitory concentration values in μg/ml.

Table 3. Cumulative percentage susceptibility of staphylococcal and streptococcal species (109 organisms) increasing concentrations of BL-S786 and 6 other parenteral cephalosporins.

Organism (#)	Cephalosporin	Cumulative % inhibited at MIC (μg/ml) of									
		≤0.06	0.125	0.25	0.5	1	2	4	8	16	32
S. aureus (25) methicillin sensitive	786[a]						28	80	100		
	CMD			24	54	86	100				
	CZ			22	58	88	98		100		
	COX			2			6	98	100		
	CLD	38	58	86	90	98	100				
	CF		8	60	94	100					
	CD					6	58	92	94	100	
S. aureus (10) methicillin resistant	786							5	15	20	35
	CMD						10	50	90	100	
	CZ						5	15	35	65	90
	COX								10	60	90
	CLD						35	80	100		
	CF					20	50	75	80	100	
	CD							5	10	20	35
S. epidermidis (18)	786					8	69	81	92	100	
	CMD		19	69	81	92	100				
	CZ		4	54	73	85	92	100			
	COX				4	19	65	85	100		
	CLD	73		81	85	100					
	CF	4	58	69	88	96		100			
	CD			12	19	23	77		92	100	
S. pneumoniae (23)	786		4	78	91		95	100			
	CMD	65	87	96	100						
	CZ	74	91	96	100						
	COX				13	78	87	100			
	CLD	91	100								
	CF	22	74	91	100						
	CD				9	83	100				
S. pyogenes (22)	786			5	95	100					
	CMD	100									
	CZ	41	100								
	COX				95		100				
	CLD	100									
	CF	5	100								
	CD		50	95		100					
S. faecalis (11)	786										9
	CMD										9
	CZ									9	82
	COX										9
	CLD									55	91
	CF										82
	CD										9

a. 786=BL-S786; CMD=cefamandole; CZ=cefazolin; COX=cefoxitin; CLD=cephaloridine; CF=cephalothin; CD=cephradine.

had clinically useable antimicrobial activity for *Serratia* species. None of the cephalosporins were active against *Pseudomonas* species.

The range and median MIC values for BL-S786, cefamandole, and cefoxitin against *Enterobacter* species and indole-positive *Proteus* species are shown in Table 2. Only these three antimicrobial agents had activity against these species groups. Cefamandole and BL-S786 both effectively inhibited all *Enterobacter* species. Cefoxitin had antimicrobial activity only against *Enterobacter agglomerans*. Among the indole-positive *Proteus* species, the susceptibility was somewhat species dependent. Cefoxitin was most active with the median MIC of 4 μg/ml. Cefamandole and BL-S786 inhibited two (*Proteus morganii* and *Proteus rettgeri*) and one (*Proteus rettgeri*) species respectively.

The susceptibility of *Staphylococcus* species and *Streptococcus* species to increasing concentrations of BL-S786 and six cephalosporins are shown in Table 3. Cephaloridine was most active against the gram-positive cocci tested. BL-S786 was among the least active cephalosporins having an efficacy similar to cephradine. The antistaphylococcal activity rank of the investigational cephalosporins was cefamandole > cefoxitin > BL-S786. Against the *Streptococcus* species, cefoxitin was least effective and BL-S786 ranked behind cefamandole. None of the parenteral antibiotics effectively inhibited *Streptococcus faecalis* at clinically useable concentrations.

Table 4 summarizes the *Haemophilus influenzae* and the *Neisseria* species MIC results for BL-S786 and six other cephalosporins. BL-S786 inhibited 44% of the *N. gonorrhoeae* isolates at the lowest concentration tested ($\leq$0.06 μg/ml) and 100% at 2 μg/ml. Cefamandole was most active against the

Table 4. MIC results of BL-S786 and other parenteral cephalosporins against *Neisseria* species and *Haemophilus influenzae* including β-lactamase producing isolates.

Organism	No. of isolates	Antibiotic	Cumulative % inhibited at MIC of								
			$\leq$0.06	0.125	0.25	0.5	1.0	2.0	4	8	16
Neisseria gonorrhoeae	25	BL-S786	44	52		64	88	100			
		Cefamandole	52	64	92		100				
		Cephalothin	36	52	64	76	100				
		Cefoxitin	4	48	56	92	100				
		Cefazolin		4	56	68	100				
		Cephaloridine			52	56	82	100			
Neisseria meningitidis	27	BL-S786	4	53	100						
		Cefamandole	97		100						
		Cefoxitin		15	100						
		Cephalothin			51	100					
		Cefazolin			12	97	100				
		Cephaloridine				8	100				
Haemophilus influenzae (β-lactamase negative)	19	BL-S786						5	90	100	
		Cefamandole		5	90	95	100				
		Cefoxitin						95	100		
		Cephalothin				10	45	80	100		
		Cefazolin					5	55	90	100	
		Cephaloridine						35	75	95	100
		Cephradine							5	85	100
Haemophilus influenzae (β-lactamase producers)	10	BL-S786							90	100	
		Cefamandole			100						
		Cefoxitin				10	20	100			
		Cephalothin					50	100			
		Cefazolin						70	100		
		Cephaloridine						10	50	100	
		Cephradine							40	100	

gonococcus. All other cephalosporins had mean MIC values between 0.125 and 0.25 μg/ml. Similar results were obtained for meningococci. Cefamandole was very active (97% inhibited at $\leq$0.06 μg/ml) and the range of the other cephalosporin mean MIC values was higher (0.125~1.0 μg/ml). BL-S786 was the second most active compound with a mean MIC of 0.125 μg/ml. Beta lactamase producing *H. influenzae* strains had slightly lower MIC values as compared to enzyme deficient strains. Cefamandole was most active and cephradine the least active parenteral cephalosporin. BL-S786 had a mean MIC value of 2~4 μg/ml against *Haemophilus*, results similar to that of cefazolin and cephaloridine.

Table 5. MIC-MBC comparison of BL-S786 and 6 other cephalosporins for *E. coli, Proteus mirabilis, Klebsiella pneumoniae* and *Staphylococcus aureus*.

Organism (#)	Antibiotic	MBC/MIC ratios		
		1	2	4 or more
E. coli (14)	BL-S786	11	1	2
	Cefamandole	13	1	0
	Cefazolin	14	0	0
	Cefoxitin	14	0	0
	Cephaloridine	12	2	0
	Cephalothin	8	4	2
	Cephradine	8	5	1
Proteus mirabilis (14)	BL-S786	8	3	3
	Cefamandole	4	9	1
	Cefazolin	10	2	2
	Cefoxitin	10	3	1
	Cephaloridine	11	2	1
	Cephalothin	11	3	0
	Cephradine	10	3	1
Klebsiella pneumoniae (14)	BL-S786	10	4	0
	Cefamandole	9	3	2
	Cefazolin	7	3	4
	Cefoxitin	14	0	0
	Cephaloridine	8	4	2
	Cephalothin	13	1	0
	Cephradine	13	1	0
S. aureus (14)	BL-S786	8	5	1
	Cefamandole	11	3	0
	Cefazolin	10	2	2
	Cefoxitin	14	0	0
	Cephaloridine	11	2	1
	Cephalothin	13	1	0
	Cephradine	11	3	0

Table 6. MIC-MBC comparison of BL-S786, cefamandole and cefoxitin against *Enterobacter* species and indole-positive *Proteus* species.

Organism (#)	Antibiotic	MBC/MIC Ratios				
		1	2	4	8	16 or more
Enterobacter[a] (10) species	BL-S786	1	1	2	2	4
	Cefamandole	3	1	2	1	3
Indole-positive[b] (10) *Proteus* species	BL-S786[c]	1	0	0	2	5
	Cefamandole	2	1	2	1	4
	Cefoxitin	6	2	1	1	0

a. Includes *Ent. cloacae* (4), *Ent. aerogenes* (4) and *Ent. agglomerans* (2). Cefoxitin omitted due to lack of antimicrobial activity among *Enterobacter* species.

b. Includes *Proteus rettgeri* (7), *Proteus morganii* (2) and one strain of *Proteus vulgaris*.

c. *Proteus morganii* strains (2) were not tabulated due to inactivity of this antibiotic.

MIC-MBC Comparisons

Tables 5 and 6 show 76 MIC-MBC comparisons for BL-S786 and six other cephalosporins. Against *E. coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *S. aureus*, 93% of the cephalosporin MIC results were the same as the MIC or within 1 dilution. BL-S786 had six of fifty-six (10.7%) of the MBC values 4 or more fold higher than the MIC. Cefazolin most often had elevated MBC results, while cefoxitin showed the least MIC-MBC variation.

A more marked MBC variation was obtained for the cephalosporins among the *Enterobacter*

Table 7. Effect of inoculum size on the MIC of BL-S786 and 6 other parenteral cephalosporins.

Organism (#)	Antibiotic	10^3 Organisms/ml MIC (μg/ml)		10^5 Organisms/ml MIC (μg/ml)		10^7 Organisms/ml MIC (μg/ml)	
		Range	Median	Range	Median	Range	Median
E. coli (16)	786[c]	0.12~2	0.5	0.12~4	0.5	4~>32	8
	CMD	0.12~4	0.5	0.12~8	0.5	2~>32	4
	CZ	0.5~4	1	0.5~8	1	4~32	8
	COX	1~32	4	1~32	4	2~>32	8
	CLD	1~8	2	2~16	2	4~>32	4
	CF	1~32	4	2~>32	8	16~>32	>32
	CD	2~32	8	4~32	8	16~>32	>32
Klebsiella pneumoniae (15)	786	0.25~4	0.25	0.25~4	0.5	2~>32	>32
	CMD	0.25~16	0.5	0.5~8	1	2~>32	>32
	CZ	1~8	1	1~8	1	4~>32	16
	COX	2~>32	2	2~>32	4	4~>32	16
	CLD	2~16	4	4~16	4	4~>32	8
	CF	1~>32	2	2~>32	4	8~>32	16
	CD	4~32	8	4~>32	8	8~>32	>32
Proteus mirabilis (16)	786	0.12~0.5	0.25	0.25~2	0.5	0.5~>32	4
	CMD	0.25~1	0.5	0.25~16	0.5	2~>32	8
	CZ	2~4	4	2~16	4	2~>32	8
	COX	2~4	2	2~32	4	2~32	16
	CLD	4~8	4	4~16	8	4~>32	16
	CF	2~4	4	2~8	4	2~>32	16
	CD	8~16	8	8~>32	16	32~>32	>32
Indole-positive *Proteus* spp. (10)[a]	786	0.06~>32	1	0.06~>32	>32	4~>32	>32
	CMD	0.06~16	0.5	0.06~>32	16	32~>32	>32
	CZ	0.12~>32	8	0.25~>32	>32	>32	>32
	COX	1~32	2	2~32	8	4~>32	16
	CLD	1~>32	32	4~>32	>32	>32	>32
	CF	0.5~>32	16	2~>32	>32	>32	>32
	CD	2~>32	16	4~>32	>32	>32	>32
Enterobacter spp. (15)[b]	786	0.12~>32	4	0.12~>32	4	>32	>32
	CMD	0.12~>32	2	1~>32	4	16~>32	>32
	CZ	1~>32	16	1~>32	>32	8~>32	>32
	COX	2~>32	>32	2~>32	>32	16~>32	>32
	CLD	2~>32	>32	2~>32	>32	8~>32	>32
	CF	1~>32	>32	1~>32	>32	16~>32	>32
	CD	4~>32	32	8~>32	>32	16~>32	>32
Staphylococcus aureus (16)	786	1~16	2	2~32	4	4~32	8
	CMD	0.12~0.5	0.5	0.25~2	0.5	0.25~32	1
	CZ	0.25~1	0.25	0.25~4	0.5	0.5~4	1
	COX	2~8	4	2~8	4	2~8	4
	CLD	0.06~0.25	0.06	0.06~1	0.125	0.06~4	0.25
	CF	0.125~0.5	0.125	0.125~1	0.25	0.25~2	0.5
	CD	1~8	2	2~16	2	2~32	8

a. Includes *Proteus rettgeri* (7), *Proteus morganii* (2) and one strain of *Proteus vulgaris*.

b. Includes *Enterobacter cloacae* (7), *Enterobacter aerogenes* (6) and *Enterobacter agglomerans* (2).

c. 786=BL-S786, CMD=cefamandole; CZ=cefazolin; COX=cefoxitin; CLD=cephaloridine; CF=cephalothin; CD=cephradine.

species and indole-positive *Proteus* species tested (Table 6). Only BL-S786 and cefamandole had antimicrobial activity against both of these groups. Only 20% and 40% of the MBC results were within a 2-fold dilution of the MIC for BL-S786 and cefamandole respectively. Cefoxitin, active only against indole-positive *Proteus* species, demonstrated a close MIC-MBC correlation.

Inoculum Size MIC Comparisons

The inoculum size effects on MIC results were studied on 103 organisms at 10^3, 10^5, and 10^7 colony forming units/ml (Table 7). The cephalosporin MICs for the *E. coli*, *K. pneumoniae*, and *P. mirabilis* strains remain constant or only increased two fold by raising the inoculum size from 10^3 to 10^5 CFU/ml. However, a marked change was encountered at 10^7 CFU/ml, where the MIC results increased 2 to greater than 64-fold. BL-S786 had MIC increases 16-fold for *E. coli*, greater than 64-fold for *K. pneumoniae*, and 8-fold for *P. mirabilis*. Comparable results were found for cefamandole. BL-S786, cefamandole, and cephradine were ineffective against *K. pneumoniae* at 10^7 CFU/ml. All tested cephalosporins had some activity against indole-positive *Proteus* species at 10^3 CFU/ml. However, at 10^5 CFU/ml inoculum size only cefamandole and cefoxitin remained effective. Only cefoxitin was active at 10^7 CFU/ml, the median MIC only rose from 8 to 16 μg/ml. BL-S786 and cefamandole were most active against *Enterobacter* species at 10^3 and 10^5 CFU/ml. This inhibitory activity was lost at the highest inoculum size.

All cephalosporins remained active against *S. aureus* at all three inoculum sizes. Cefoxitin MIC values (median) remained unchanged while other cephalosporins increased 2- to 4-fold.

Additional studies were performed on seven methicillin-resistant *S. aureus* and eight non-enterococcus group D streptococci (not tabulated). BL-S786 median MIC results at each inoculum size were consistently 8-fold higher for methicillin-resistant strains of *S. aureus* compared to methicillin-sensitive strains in Table 7. Insignificant inoculum size effects were found among the *S. bovis* and *S. durans* strains. Cefazolin and cephaloridine median MICs were unchanged, and the remaining five cephalosporins MICs increased 2-fold from 10^5 to 10^7 CFU/ml inoculum size.

Discussion

BL-S786 has been described as a semisynthetic parenteral cephalosporin possessing a wider and more active antimicrobial spectrum currently available agents[1,2]. This study confirms that observation and adds additional comparative data with cephradine, cefamandole, and cefoxitin. In comparisons with the latter cephalosporins, BL-S786 antimicrobial activity was most similar to cefamandole though BL-S786 was the most effective cephalosporin tested against *K. pneumoniae, C. diversus,* and *Salmonella* species. BL-S786 had an antimicrobial activity against the *Enterobacter* species, a feature like cefamandole[4], and cefuroxime[3,6]. However, cefamandole was approximately 4-fold more active against the *Enterobacter* species than BL-S786 (Table 2). *Proteus rettgeri* isolates were inhibited by BL-S786, but the other indole-positive *Proteus* species and *Serratia marcescens* were resistant.

Haemophilus influenzae was moderately sensitive to BL-S786. The mean MIC was 4 μg/ml, a value comparable to cefazolin, cephaloridine and cephradine. Cefamandole was the most active cephalosporin against beta lactamase producing and deficient *Haemophilus* species confirming previous studies[3,5].

BL-S786 MICs for the gonococcus had a bimodal distribution with all isolates inhibited by 2 μg/ml. The lowest mode was at <0.06 μg/ml, the second mode at 1 μg/ml. The pattern was identical to that of cephalothin. Cefamandole had the best activity with closely associated modes ≤ 0.6 μg/ml and 0.25 μg/ml. BL-S786 was very active against *N. meningitidis*. All isolates tested were inhibited by 0.25 μg/ml of BL-S786 and no evidence of bimodal MIC distribution was identified.

Of particular importance were the findings of the MBC and inoculum size MIC comparisons. The MBC values in 93% of the organisms were unchanged or only 2-fold increased over the MIC values (Table 5). The BL-S786 and cefamandole inhibitory antimicrobial activity against *Enterobacter* and indole-positive *Proteus* were markedly reduced when bactericidal values (MBCs) were determined. Only 13~30% of the BL-S786 and cefamandole MBCs were equal to the MIC results. The majority of the MBC results were ≥8-fold higher than the MIC result. Similarly the highest inoculum size of 10^7 CFU/ml negates the favorable BL-S786 and cefamandole MICs against the *Enterobacters* and indole-positive *Proteus* species. Only cefoxitin remains effective against *Proteus morganii*, *Proteus vulgaris*, and *Proteus rettgeri* when MBCs were examined and the inoculum size increased. Cefoxitin was inactive against the *Enterobacter* species.

BL-S786 was less effective for gram-positive cocci than currently available cephalosporins, except cephradine. *S. faecalis* and *Pseudomonas* species were resistent to BL-S786 and all other cephalosporins tested.

BL-S786 appeared to be a promising new semisynthetic cephalosporin. The *in vitro* antimicrobial characteristics were generally superior to currently available cephalosporins and comparable to investigational drugs such as cefamandole, cefuroxime, and cefoxitin. Pharmacokinetic studies in animals and in humans were favorable and include prolonged biologic half life, high serum levels, active urinary excretion and relatively low ED-50 results[2].

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References

1) JONES, R. N.; P. C. FUCHS, T. L. GAVAN, E. H. GERLACH, A. L. BARRY & C. THORNSBERRY: BL-S786, a new parenteral cephalosporin. I. A collaborative *in vitro* susceptibility comparison to cephalothin against 5,762 clinical bacterial isolates. J. Antibiotics 30: 576~582, 1977
2) LEITNER, F.; M. MISIEK, T. A. PURSIANO, R. E. BUCK, D. R. CISHOLM, R. G. DEREGIS, Y. H. TSAI & K. E. PRICE: Laboratory evaluation of BL-S786, a cephalosporin with broad-spectrum antimicrobial activity. Antimicr. Agents & Chemoth. 10: 426~435, 1976
3) BARRY, A. L.; C. THORNSBERRY, R. N. JONES, P. C. FUCHS, T. L. GAVAN & E. H. GERLACH: Cefuroxime, *in vitro* comparison to six other parenteral cephalosporins. (In Press). 1977
4) STEERS, E. E.; E. L. FOLTZ & B. S. GRAVES: An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. & Chemoth. 9: 307~311, 1959
5) JONES, R. N. & P. C. FUCHS: Comparison of *in vitro* antimicrobial activity of cefamandole and cefazolin with cephalothin against over 8,000 clinical bacterial isolates. Antimicr. Agents & Chemoth. 9: 1066~1069, 1976
6) EYKYN, S.; C. JENKINS, A. KING & I. PHILLIPS: Antibacterial activity of cefuroxime, a new cephalosporin antibiotic, compared with that of cephaloridine, cephalothin, and cefamandole. Antimicr. Agents & Chemoth. 9: 690~695, 1976