BL-S786, A NEW PARENTERAL CEPHALOSPORIN. I

A COLLABORATIVE *IN VITRO* SUSCEPTIBILITY COMPARISON TO CEPHALOTHIN AGAINST 5,762 CLINICAL BACTERIAL ISOLATES

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The *in vitro* activity of Compound BL-S786 was compared with that of cephalothin against 5,762 clinical isolates by the microdilution broth method. BL-S786 demonstrated a broader spectrum and a significantly lower MIC against the *Enterobacteriaceae*. Although greater susceptibility to BL-S786 than to cephalothin was exhibited by *Serratia marcescens*, *Proteus morganii* and *Proteus vulgaris*, these three species were generally resistant to both drugs. By contrast, the staphylococci were significantly more susceptible to cephalothin than to BL-S786. Resistance to both drugs was demonstrated by *Pseudomonas aeruginosa* and other pseudomonads, enterococci and *Bacteroides fragilis*.

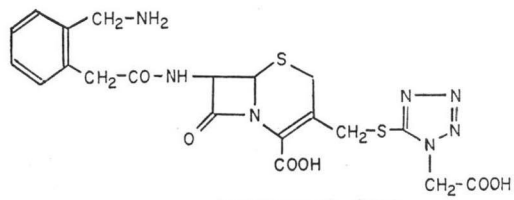
Compound BL-S786, 7-[α-(2-aminomethylphenyl)acetamido]-3-[(1-carboxymethyltetrazol-5-ylthio)-methyl]-3-cephem-4-carboxylic acid, is a new semisynthetic cephalosporin developed for parenteral administration.[1] BL-S786 joins a number of other investigational parenteral cephalosporins and cephamycins including cephamandole, cefoxitin, and cefuroxime, offering wider and/or more active antimicrobial activity than currently popular cephalothin.[2~7] This collaborative study compares the *in vitro* antimicrobial activity of BL-S786 with that of cephalothin on a large number of clinical bacterial isolates from three geographic areas.

Materials and Methods

Antimicrobial Agents:

BL-S786 was supplied by Bristol Laboratories, Syracuse, New York. Cephalothin laboratory standard powder was furnished by Lilly Research Laboratories, Eli Lilly & Co, Indianapolis, Indiana.

Fig. 1 Chemical structure of Compound BL-S786



Compound BL-S786

Bacterial Isolates:

The 5,762 organisms used in the study were consecutive clinical strains isolated at the Clinical Microbiology Laboratories of the Cleveland Clinic (Cleveland, Ohio), Kaiser Foundation Hospitals (Portland, Oregon), St. Francis Hospital (Wichita, Kansas), and St. Vincent Hospital (Portland, Oregon). A total of 5,646 aerobic and facultative anaerobic isolates were tested with an additional 116 strict anaerobic organisms. Each isolate was processed and identified by standardized procedures using 10~24 biochemical tests. Identifications were performed by the replicator-plate method described by FUCHS[8] or the API system. Additional phage typing, serologic typing, fluorescent antibody identifications, counter-current electrophoresis procedures, and antimicrobial agent susceptibility patterns were used where needed.

Antimicrobial Susceptibility Testing:

Minimum inhibitory concentrations (MICs) for all antimicrobial agents were determined by the microdilution broth method. MUELLER-HINTON broth (Difco) was used commercially dispensed in plastic trays (Micro Media Systems, Campbell, California), or dispensed in the participating laboratories using the MIC-2000 (Cooke Instruments, Alexandria, Virginia). The antimicrobial agents were dispensed in 100-μl volumes with a total of seven 2-fold dilutions of BL-S786 and cephalothin, ranging from 0.5 μg/ml to 32 μg/ml. Automatic inoculators manufactured by Micro Media Systems and Cooke Instruments were used to deliver 5 and 1 μg to each of the wells, respectively. Final inoculum size was adjusted to 5×10^5 organism/ml.

Minimum inhibitory concentration endpoints were defined as the lowest well concentration totally inhibiting organism growth (clear well), after 15~18 hours of incubation at 35°C in forced air incubators.

Quality control strains having known MIC values were tested in parallel with the study strains. These quality control organisms included *E. coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Streptococcus faecalis* (ATCC 29212). Summary data of the quality control results during the study interval are found in Table 1.

Susceptibility testing of *Streptococcus pneumoniae*, several beta hemolytic streptococci, and *Haemophilus* species were performed in MUELLER-HINTON broth supplemented with 5% peptic digest of horse cells[9]. The anaerobic susceptibility testing utilized brain-heart infusion broth containing 0.1 μg/ml menadione and 0.01 μg/ml of hemin. The anaerobic identification and susceptibility procedures were performed as previously described[10].

The KOLMOGOOV-SMIRNOV two samples significance test was used for statistical analysis of the susceptibility results.

Results

Quality Control of Susceptibility Test Method

Four organisms were used daily to quality control the microdilution broth susceptibility test

Table 1. Quality control organism results for the microdilution broth minimal inhibitory concentration method used by the four collaborating laboratories (three organisms).

Organism	Antibiotic	Expected MIC	Variation from expected dilution		
			0	±1	>±1
E. coli ATCC 25922	786	0.5	170	35	3
	CF	8	148	78	2
S. aureus ATCC 25923	786	2	89	74	1
S. faecalis ATCC 29212	CF	32	162	23	1
Total (%)	—	—	569(72.4)	210(26.7)	7(0.9)

methods. A total of 786 MIC endpoint results were made by the four collaborating laboratories during the test interval. No variation from the expected MIC was shown for 72.4% of results. A total of 99.1% of the MICs were within the ±1 dilution range.

BL-S786 Antimicrobial Activity against the Enterobacteriaceae

The accumulative percentages of *Enterobacteriaceae* inhibited by increasing concentrations of BL-S786 and cephalothin are shown in Table 2. BL-S786 was more active than cephalothin against *E. coli, Klebsiella pneumoniae, Citrobacter diversus*, and *Proteus mirabilis*. At 1 μg/ml BL-S786 inhibited 84% of *E. coli* strains in contrast to 4% for cephalothin. Similar percentage differences were found for *Klebsiella* species, *Citrobacter diversus*, and *Proteus mirabilis* strains. Nearly all of these species (95~98%) were inhibited by BL-S786 at 16 μg/ml or less.

Table 2. Comparison of BL-S786 and cephalothin (CF) MIC's for 3,634 clinical *Enterobacteriaceae* isolates

Organism (#)	Antibiotic	Cumulative % susceptible @ MIC of							
		≤0.5	1	2	4	8	16	32	>32
E. coli	BL-S786	67	84	90	94	96	98	99	100
(1904)	CF		4	14	49	78	91	95	100
Klebsiella pneumoniae	BL-S786	71	83	93	96	98			100
(560)	CF		2	34	75	91	96	97	100
Klebsiella ozaenae	BL-S786	55	64	73		100			
(11)	CF		9	36	73		91		100
Enterobacter cloacae	BL-S786	5	9	20	37	61	70	81	100
(196)	CF		1			2	6	11	100
Enterobacter aerogenes	BL-S786	14	41	66	76	82	83	84	100
(125)	CF			3	7		25	51	100
Enterobacter agglomerans	BL-S786	35	43	65	70	76	81		100
(37)	CF		8	15	36	49	67	74	100
Enterobacter hafniae	BL-S786	8		58	83	92	100		
(12)	CF						25		100
Serratia marcescens	BL-S786			3	5	8	18	22	100
(95)	CF							5	100
Serratia species [a]	BL-S786		40		60		80		100
(5)	CF							20	100
Proteus mirabilis	BL-S786	86	95	97		98		99	100
(432)	CF		3	61	92	96	97	98	100
Proteus morganii	BL-S786	9	11			13	16	31	100
(45)	CF			7		9		11	100
Proteus rettgeri	BL-S786	23	46	65		69	81		100
(26)	CF			4		7	18	25	100
Proteus vulgaris	BL-S786			7	14			21	100
(14)	CF							7	100
Providencia species	BL-S786	71							100
(7)	CF			29	57		71		100
Citrobacter freundii	BL-S786	17	19	46	71	83	87		100
(63)	CF			5	11	21	43	57	100
Citrobacter diversus	BL-S786	83	90	93	95			100	
(40)	CF		2	67	88	90	93		100
Salmonella enteritidis	BL-S786	75			81	94			100
(16)	CF		39	61			72	94	100
Yersinia enterocolitica	BL-S786			8	20	44	76	100	
(25)	CF					4	28	48	100
Others [b]	BL-S786	71	81	86	100				
(21)	CF		33	48	71	86	90	100	

a. Includes *Serratia liquefaciens* (4) and one strain of *Serratia rubidea*.

b. Includes enteropathogenic *E. coli* (12), *Edwardsiella tarda* (5), *Shigella* species (3), and one strain of *Arizona arizonae*.

The *Enterobacter* species and *Citrobacter freundii* were also more susceptible to BL-S786. The mean MIC value for all strains was 1~8 μg/ml as compared to 32 μg/ml or greater for cephalothin. The *Enterobacter* species were 70~100% inhibited by 16 μg/ml or less. Only a minimal species variation in susceptibility was encountered with *Enterobacter cloacae* being most resistant to BL-S786.

In contrast, indole-positive *Proteus* species and *Providencia* species demonstrated marked species variation in susceptibility.

BL-S786 showed minimal inhibition of *Serratia marcescens* isolates. Only 18% were inhibited at 16 μg/ml or less. A good inhibitory response was encountered with other *Serratia* species. BL-S786 antimicrobial activity against *Proteus rettgeri* and the *Providencia* species was excellent. BL-S786 inhibited 23% of the *Proteus rettgeri* strains at 0.5 μg/ml and 81% at 16 μg/ml. This contrasted to only 18% inhibition by cephalothin at 16 μg/ml. *Providencia* species showed 71% inhibition at the lowest tested concentration of 0.5 μg/ml. This represented a 32-fold BL-S786 activity advantage over cephalothin. Minimal BL-S786 activity was noted against the *Proteus morganii* and *Proteus vulgaris* species.

Salmonella enteritidis strains showed 75% inhibition at 0.5 μg/ml of BL-S786. Cephalothin concentrations of 16 μg/ml were needed to inhibit a similar percentage (72%) of the *Salmonella* isolates. BL-S786 was approximately 4-fold more active than cephalothin for the 25 *Yersinia enterocolitica* species tested.

BL-S786 Antimicrobial Activity against Gram-positive Cocci

Table 3 compares BL-S786 and cephalothin against 1,265 gram-positive cocci. Among the gram-

Table 3. MIC Comparison of BL-S786 and cephalothin (CF) for 1,265 clinical Gram-positive cocci

Organism (#)	Antibiotic	Cumulative % susceptible @ MIC of							
		≤0.06	0.125	0.25	0.5	1[a]	2	4	>4
Staphylococcus aureus (608)	BL-S786			1	1	4	33	88	100
	CF		17	89	98	99			100
Staphylococcus epidermidis (250)	BL-S786			3	6	17	56	82	100
	CF		29	71	91	96	98		100
Micrococcus species (3)	BL-S786					100			
	CF					100			
Streptococcus, beta hemolytic, not gr. A, B, or D (70)	BL-S786	9	10	27	71	77	87	93	100
	CF		54	64	71	94			100
Streptococcus agalactiae (33)	BL-S786	3		21	69	78	93	100	
	CF					100			
Streptococcus pyogenes (32)	BL-S786	6	24	66	90	93		100	
	CF					100			
Streptococcus viridans (32)	BL-S786	22	37	56	78	81	89	93	100
	CF		50	88				94	100
Streptococcus pneumoniae (4)	BL-S786			25	50	100			
	CF		25	50		100			
Streptococcus faecalis (181)	BL-S786						2		100
	CF						1		100
Streptococcus faecium (15)	BL-S786							7	100
	CF					6	12	18	100
Streptococcus bovis (9)	BL-S786				11	22	33	89	100
	CF			8		92			100
Streptococcus durans (7)	BL-S786				14	43		57	100
	CF					75			100
Other Streptococci [b] (21)	BL-S786	10		19	33	48	81	86	100
	CF		29	71		86		90	100

a. Lowest tested cephalothin concentration for *S. agalactiae* and *S. pyogenes*.

b. Includes gamma hemolytic streptococci (6), *S. mutans* (4), two strains each of *S. equinus*, *S. mitis*, *S. dysgalactiae*, *S. sanguinus* and *S. anginosus*, and one strain of *S. avium*.

positive cocci tested cephalothin was generally more active. For *Staphylococcus aureus* and *Staphylococcus epidermidis* the BL-S786 mean MIC was between 2 and 4 μg/ml. This contrasts to a cephalothin MIC mean value of 0.125 to 0.25 μg/ml for both staphylococcal species. The non group D streptococcal species were susceptible to BL-S786, but at higher concentrations than cephalothin. All but 6 isolates were inhibited by BL-S786 concentrations of 4 μg/ml or less. Cephalothin was consistently 2~8 fold more active than the new compound.

Lancefield group D streptococci were generally resistant to both cephalosporin agents tested. This was particularly true of *S. faecalis* and *S. faecium*. Cephalothin was at least 4-fold more active than BL-S786 against *S. bovis* and *S. durans*.

BL-S786 Antimicrobial Activity against Non-Enterobacteriaceae Gram-negative Bacteria

BL-S786 and cephalothin showed similar activity against most of the species listed in Table 4. Both cephalosporins were inactive against *A. calcoaceticus* subspecies *anitratus*, *Pseudomonas aeruginosa*, *Pseudomonas* species, *Achromobacter* species, *Flavobacterium* species, *Alcaligenes* species and group VE-1. BL-S786 and cephalothin were similarly effective for *Moraxella* species and *Neisseria meningitidis*.

Among the 19 strains of *Acinetobacter calcoaceticus* subspecies *lwoffi* BL-S786 was approximately 2-fold more active than cephalothin. *Haemophilus influenzae* and *Haemophilus* species isolates had a BL-S786 mean MIC of 4~8 μg/ml, with the cephalothin mean MIC of only 1~2 μg/ml.

Table 4. Comparison of BL-S786 and cephalothin (CF) MIC's for 747 clinical non-*Enterobacteriaceae* Gram-negative organisms including *N. meningitidis*

Organism (#)	Antibiotic	Cumulative % susceptible @ MIC of							
		≤0.5	1	2	4	8	16	32	>32
Acinetobacter calcoaceticus subsp. *anitratus* (57)	BL-S786	2			4		5	19	100
	CF			2		4		7	100
Acinetobacter calcoaceticus subsp. *lwoffi* (19)	BL-S786	5			15	25	35	40	100
	CF		5			15	20	35	100
Haemophilus influenzae (148)	BL-S786	21		28	55	90	97	98	100
	CF		28	66	85	94	97	99	100
Haemophilus species (11)	BL-S786	9	18		36	73	91		100
	CF		18	64	73	82	91		100
Moraxella species (15)	BL-S786	20	40	47		53	60	67	100
	CF		47	53	60			67	100
Neisseria meningitidis (10)	BL-S786	70				100			
	CF	70	90			100			
Pasteurella multocida (3)	BL-S786	33			100				
	CF		100						
Pseudomonas aeruginosa (404)	BL-S786							1	100
	CF		1						100
Pseudomonas species [a] (59)	BL-S786	3			7	8	10		100
	CF		2	3			7	14	100
Other, Group I [b] (10)	BL-S786	40		50		60	70	80	100
	CF		50	60	70				100
Other, Group II [c] (11)	BL-S786								100
	CF								100

a. Includes *Pseudomonas stutzeri* (21), *P. maltophilia* (8), *P. alcaligenes* (8), *Pseudomonas* species NOS (7), *P. cepacia* (5), *P. fluorescens* (5), *P. putida* (2) and one strain each of *P. acidovorans*, *P. putrefaciens* and *P. testosteroni*.

b. Includes *Aeromonas hydrophila* (4), *Aeromonas shigelloides* (2), HB-5 (2), and one strain each of *Eikenella corrodens* and *Actinobacillus* species.

c. Includes *Achromobacter* species (4), *Flavobacterium* species (3), *Alcaligenes* species (2) and Group VE-1 (2).

BL-S786 Antimicrobial Activity against Anaerobic Isolates

Table 5 shows the BL-S786 antimicrobial activity against 116 clinical isolates of strict anaerobes. Only 8% of the tested *Bacteroides fragilis* strains were inhibited at tested concentrations (32 μg/ml). Three strains of *Eubacterium* species showed similar relative resistance to BL-S786

However, 100% of the remaining organisms were susceptible to 32 μg/ml or less of BL-S786 and the mean MIC for the *Clostridium* species, gram-positive cocci, *Bacteroides* and *Fusobacterium* species and the gram-positive non-sporulating bacilli were less than 0.5 μg/ml.

Table 5. Minimal inhibitory concentrations of BL-S786 for 116 clinical anaerobic isolates.

Organism (#)	Cumulative % inhibited at MIC of				
	≤0.5	2	8	32	>32
Bacteroides fragilis (48)			2	8	100
Clostridium species[a] (44)	59	82	98	100	
Gram-positive cocci[b] (10)	70	80	100		
Bacteroides & Fusobacterium species (7)	71	86		100	
Gram-positive non-sporulating bacilli[c] (4)	75	100			
Eubacterium species (3)			33	67	100

a. Includes *C. perfringens* (33), *C. ramosum* (3), *C. butyricum* (2), *C. paraputrificum* (2) and one isolate each of *C. septicum, C. oroticum, C. lentoputrescens* & *C. sordellii.*

b. Includes *Peptococcus asaccharolyticus* (3), *Pc. variabilis* (2) and one isolate each of *Peptostrepococcus intermedius, Ps. anaerobius, Ps. magnus, Ps. morbillorum* and *Ruminococcus bromii.*

c. Includes *Propionibacterium acnes* (2), *Lactobacillus acidophilus* (1) and *L.* species (1).

Discussion

This study demonstrated BL-S786 to be highly effective against recent clinical bacterial isolates. Also shown was a broader spectrum and higher antimicrobial activity particularly among the *Enterobacteriaceae* compared to that of cephalothin. Similar results were reported by LEITNER, *et al.* using cephaloridine, cefazolin, and cephalothin as reference cephalosporins[1].

BL-S786 possesses potent antimicrobial activity against commonly encountered clinical *Enterobacteriaceae*, especially *E. coli, Klebsiella* species, *Proteus mirabilis, Citrobacter diversus* and *Salmonella enteritidis.* All the above species had mean BL-S786 MIC values less than 0.5 μg/ml compared to 1~8 μg/ml for cephalothin. *E. coli* ($P = < 0.001$), *Klebsiella pneumoniae* ($P = < 0.001$) and *Proteus mirabilis* ($P = < 0.001$) were statistically more inhibited at concentrations up to 16, 4 and 8 μg/ml respectively.

In addition, *Enterobacter* species, *Proteus rettgeri* and *Providencia* species were inhibited by BL-S786. A highly significant increase in antimicrobial activity was noted for BL-S786 compared to cephalothin for *Enterobacter cloacae* ($P = < 0.001$) and *Enterobacter aerogenes* ($P = < 0.001$), at all tested concentrations. This activity was comparable to that previously reported for cefamandole and cefuroxime[4,11]. Only *Serratia marcescens*, though significantly more active ($P = < 0.01$), *Proteus morganii* and *Proteus vulgaris* strains were consistently resistant to achievable levels of BL-S786.

Cephalothin was 2- to 4-fold more active than BL-S786 against staphylococci and streptococci. This difference achieved statistical significance ($P = < 0.001$) against *S. aureus* isolates at less than or equal to 2 μg/ml. Neither cephalosporin was effective against *Streptococcus faecalis* or *Streptococcus faecium.*

Both BL-S786 and cephalothin were equally effective against the non-*Enterobacteriaceae* gram-negative organisms tested. *Acinetobacter calcoaceticus* var. *anitratus, Pseudomonas aeruginosa, Pseudomonas* species and several other rarely encountered gram-negative rods were resistant. All other species tested showed varying degrees of sensitivity, including *Haemophilus influenzae, Moraxella*

species, *Neisseria meningitidis*, and *Acinetobacter calcoaceticus* var. *lwoffi*. Like other cephalosporins BL-S786 was ineffective against *Bacteroides fragilis* (8% inhibited at 32 μg/ml), but was quite active against other anaerobic strains[2,12].

BL-S786 in direct *in vitro* comparison with cephalothin on current clinical isolates demonstrates definite potential as a parenteral antimicrobic. Additional *in vitro* comparisons with other investigational and available cephalosporins, determinations of disc diffusion susceptibility criteria, and animal infection studies are needed. These studies form the content of future communications.

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