ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1976, p. 426-435
Vol. 10, No. 3

Printed in U.S.A.

Laboratory Evaluation of BL-S786, a Cephalosporin with Broad-Spectrum Antibacterial Activity

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Received for publication 3 May 1976

Biological and physicochemical properties of BL-S786 were compared with those of cephalothin, cephaloridine, and cefazolin. With few exceptions, BL-S786 was more active than the reference compounds against major gram-negative pathogenic species and its antibacterial spectrum was broader than that of cephalosporins currently available for clinical use. Although BL-S786 was generally less active than the control cephalosporins against gram-positive pathogens, it inhibited their growth at concentrations that should readily be achieved in humans after standard parenteral dosage. *Streptococcus faecalis*, a species relatively unsusceptible to cephalosporins in general, was an exception. BL-S786 was an effective bactericidal agent for strains of various gram-negative organisms. After intramuscular administration to mice, BL-S786 achieved high concentrations in blood, and its biological half-life was longer than that of the other three cephalosporins.

BL-S786 (Fig. 1) is a new semisynthetic cephalosporin with a broad spectrum of antibacterial activity. The following is a report on biological and physicochemical properties of BL-S786 in comparison with those of cephalothin, cephaloridine, and cefazolin, three cephalosporins widely used clinically in the United States and abroad.

MATERIALS AND METHODS

Cephalosporins. BL-S786, 7-[α-(2-aminomethylphenyl)acetamido]-3-[(1-carboxymethyltetrazol-5-ylthio)methyl]-3-cephem-4-carboxylic acid, was synthesized by members of the Product Development Department, Bristol Laboratories (W. J. Gottstein, M. A. Kaplan, J. A. Cooper, V. H. Silver, S. J. Nachfolger, and A. P. Granatek, submitted for publication). Cephalothin and cephaloridine were products of Eli Lilly and Co.; cefazolin was obtained from Fujisawa Pharmaceutical Co. and Smith Kline and French Laboratories.

Bacteria. The organisms, preponderantly of recent clinical origin, were obtained from numerous sources of broad geographical distribution. Obligate anaerobes were maintained in egg meat medium (Difco); *Mycobacterium* was stored on Lowenstein medium (Jensen modification; Difco). The techniques of storing all other organisms have been described previously (1).

Antibiotic spectrum. The growth-inhibitory activity of BL-S786 and the control compounds was determined by the antibiotic dilution technique. Procedures were as follows.

(i) Aerobic organisms (excluding *Mycobacterium*). Except for *Haemophilus* and *Neisseria*, the assay was performed in Mueller-Hinton medium (Difco). For fastidious organisms, i.e., *Streptococcus, Listeria, Pasteurella, Bordetella,* and *Vibrio,* the medium was supplemented with 4% defibrinated sheep blood. The antibiotic susceptibility of *Haemophilus* and *Neisseria* was determined in GC medium base (BBL) supplemented with 1% hemoglobin (BBL) and 1% IsoVitaleX (BBL).

Overnight broth cultures or an exponentially growing culture (*Neisseria*) served as the source of inoculum. A volume of approximately 0.003 ml of the undiluted or diluted culture was applied to the surface of the antibiotic-containing agar plates with the inoculator of Steers et al. (5). Cultures of *Neisseria, Streptococcus pneumoniae, S. viridans,* and *S. pyogenes* were used without dilution; those of all other organisms were diluted 100-fold. The inoculum contained about 10^3 viable cells for *Neisseria*, 10^5 for *S. pneumoniae* and *S. pyogenes*, 10^6 for *S. viridans*, and 10^4 for all other species. The culture plates were incubated at 37°C either overnight or for 24 h (*Haemophilus*), and the minimum inhibitory concentration, i.e., the lowest concentration of antibiotic that prevents visible growth, was recorded.

(ii) *Mycobacterium tuberculosis*. Antimycobacterial activity was assayed in Dubos liquid medium (Difco). To 1 ml of antibiotic-containing medium, 3 ml of a 50-fold diluted, 10-day-old culture was added. Minimum inhibitory concentration values were determined after further incubation at 37°C for 10 days.

(iii) Obligate anaerobes. The assay was performed on solid medium, consisting of brain heart infusion broth (BBL) supplemented with 5% defibrinated sheep blood and 1% Ionagar (Colab). A volume of approximately 0.003 ml of a cell suspension, con-

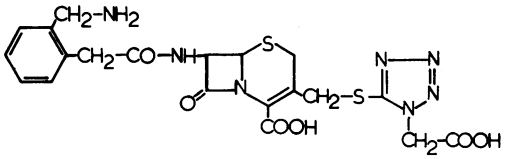

FIG. 1. *Structure of BL-S786.*

taining 10^4 viable cells, was applied to the surface of the medicated agar plates with the inoculator of Steers et al. (5). The plates were incubated at 37°C for 48 h under the oxygen-free conditions attained with a GasPak (BBL) brewer jar system.

Binding to serum proteins. The degree of binding to human serum proteins was estimated by means of the antibiotic diffusion technique of Scholtan and Schmid (4). The assays were performed in 95% pooled human serum. The range of cephalosporin concentrations was as follows: 3 to 20 μg/ml for BL-S786; 1 to 20 μg/ml for cephalothin; 0.5 to 5 μg/ml for cephaloridine; and 4 to 100 μg/ml for cefazolin.

Stability in solution. Stability in solution was determined at 37°C in 0.005 M phosphate buffer, pH 7.4. The initial antibiotic concentration was 0.2 mg/ml for cephalothin, 0.6 mg/ml for BL-S786 and cefazolin, and 2 mg/ml for cephaloridine. Residual antibiotic activity was determined periodically over a 24-h period by a turbidimetric assay procedure or by an antibiotic diffusion technique (cephalothin).

Antibiotic concentration in blood. Male Swiss-Webster mice, weighing 19 to 22 g, were given 0.2 ml of antibiotic solutions at appropriate concentrations by intramuscular injection. The vehicle was 0.01% phosphate buffer at pH 7.0. Eight animals were used for each dose level (5, 10, 20, and 40 mg/kg). Blood samples (0.03 ml) were obtained from the orbital sinuses by means of heparinized capillary tubes (Clay Adams) at 0.25, 0.5, 1, and 1.5 h after administration of the compound. Paper disks, 6.35 mm in diameter, were impregnated with the blood, and the antibiotic activity was assayed by the diffusion technique using seed agar (BBL) inoculated with *Bacillus subtilis* ATCC 6633. A standard line relating the diameter of the inhibition zone to drug concentration was obtained by assaying the compounds at known concentrations in heparinized mouse blood.

Recovery in urine. Male Sprague-Dawley rats, weighing 180 to 220 g, received a dose of 10 mg of antibiotic per kg in 0.4 ml of 0.01% phosphate buffer, pH 7.0, by intramuscular injection. Depending on the experiment, 4, 5, or 10 rats were used per compound. The animals were hydrated with 5 ml of water 0, 3, and 6 h after dosing. The rats were housed individually in metabolism cages, and urine specimens were collected over dry ice during intervals of 0 to 6 and 6 to 24 h after administration. Samples (0.03 ml) of appropriate dilutions of urine were placed on paper disks (6.35 mm in diameter), and the antibiotic activity was assayed by the diffusion technique on seed agar inoculated with *B. subtilis* ATCC 6633. A standard line relating the diameter of the inhibition zone to drug concentration was obtained by assaying the compounds at known concentrations in urine collected from untreated control animals.

Treatment of systemically infected mice. The procedures were identical to those published previously (2) except that: (i) the hog gastric mucin used in infections with *Staphylococcus aureus* no. 2 was purchased from American Laboratories, Inc., Omaha, Neb. (lot no 154163), and (ii) the medium used to suspend all other organisms contained 3% (rather than 4%) hog gastric mucin (type 1701W, Wilson Laboratories, Inc., Park Forest South, Ill.).

Miscellaneous. Methods for determining bactericidal activity and susceptibility to cell-free β-lactamase were those previously used (1).

RESULTS

Properties in vitro. (i) Antibiotic spectrum. The growth-inhibitory activity of BL-S786 and the three control cephalosporins is illustrated in Fig. 2 to 4 and Tables 1 and 2.

BL-S786 was from 2 to 16 times more active than the reference cephalosporins against *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Salmonella* sp., *Shigella* sp., and *Neisseria meningitidis* (Fig. 2 and 3). BL-S786 was also the most active of the four cephalosporins against a small number of strains of *Edwardsiella tarda, Arizona hinshawii,* and *Erwinia* sp. (Table 1). Ninety-eight percent of the *Providencia stuartii* and 78% of the *Proteus rettgeri* strains were inhibited by BL-S786 at a concentration of 16 μg/ml. At the same concentration, cefazolin inhibited 23% and 44% of the strains, respectively, whereas the percentage of strains inhibited 23 and 44% of the strains, respecnegligible for both species. Against *Enterobacter* sp. and *Citrobacter* sp., BL-S786 alone was active. Against *Haemophilus influenzae,* including nine ampicillin-resistant strains, BL-S786, cephaloridine, and cefazolin were about equally active, whereas cephalothin was more than twice as active as the other cephalosporins. *Neisseria gonorrhoeae* was equally susceptible to BL-S786, cephalothin, and cefazolin. Five strains of *Alcaligenes* sp. were susceptible and six were resistant to the four cephalosporins. *Pasteurella multocida* was highly susceptible, whereas *Proteus morganii, Serratia marcescans, Pseudomonas aeruginosa,* and *Bacteroides fragilis* were resistant to all of the cephalosporins.

BL-S786 was generally less active than the control compounds against staphylococci, streptococci, *Listeria monocytogenes,* and *Clostridium* sp. (Fig. 4 and Table 2). Nevertheless, BL-S786, at 4 μg/ml, inhibited 62 strains and, at 8 μg/ml, all 63 strains of *S. aureus.* Furthermore, BL-S786 inhibited all strains of *S. pneu-*

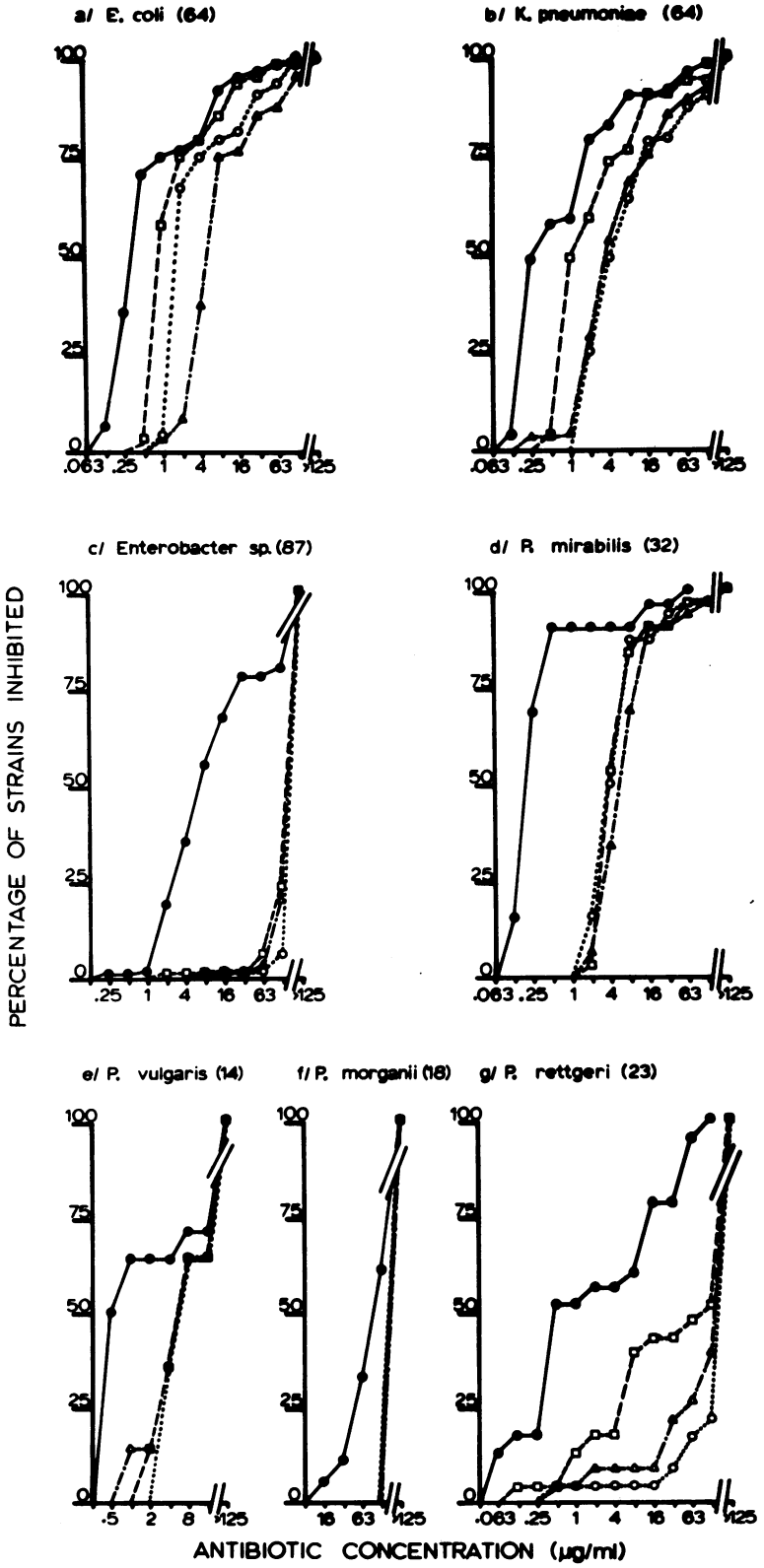

FIG. 2. *Growth-inhibitory activity of BL-S786 (●), cephalothin (△), cephaloridine (○), and cefazolin (□) against strains of various Enterobacteriaceae. The numeral in parentheses indicates the number of strains.*

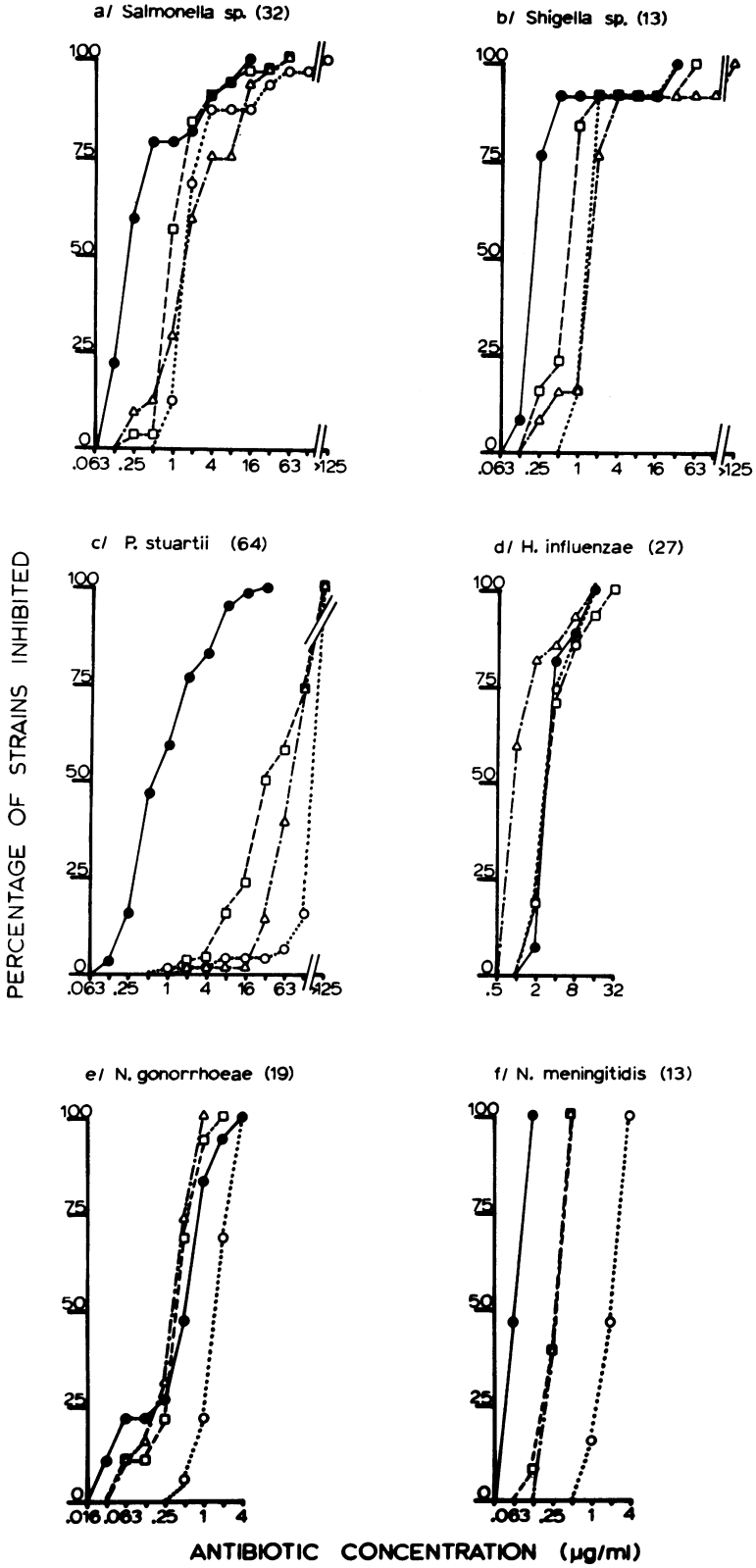

FIG. 3. *Growth-inhibitory activity of BL-S786 (●), cephalothin (△), cephaloridine (○), and cefazolin (□) against strains of various gram-negative organisms. The numeral in parentheses indicates the number of strains.*

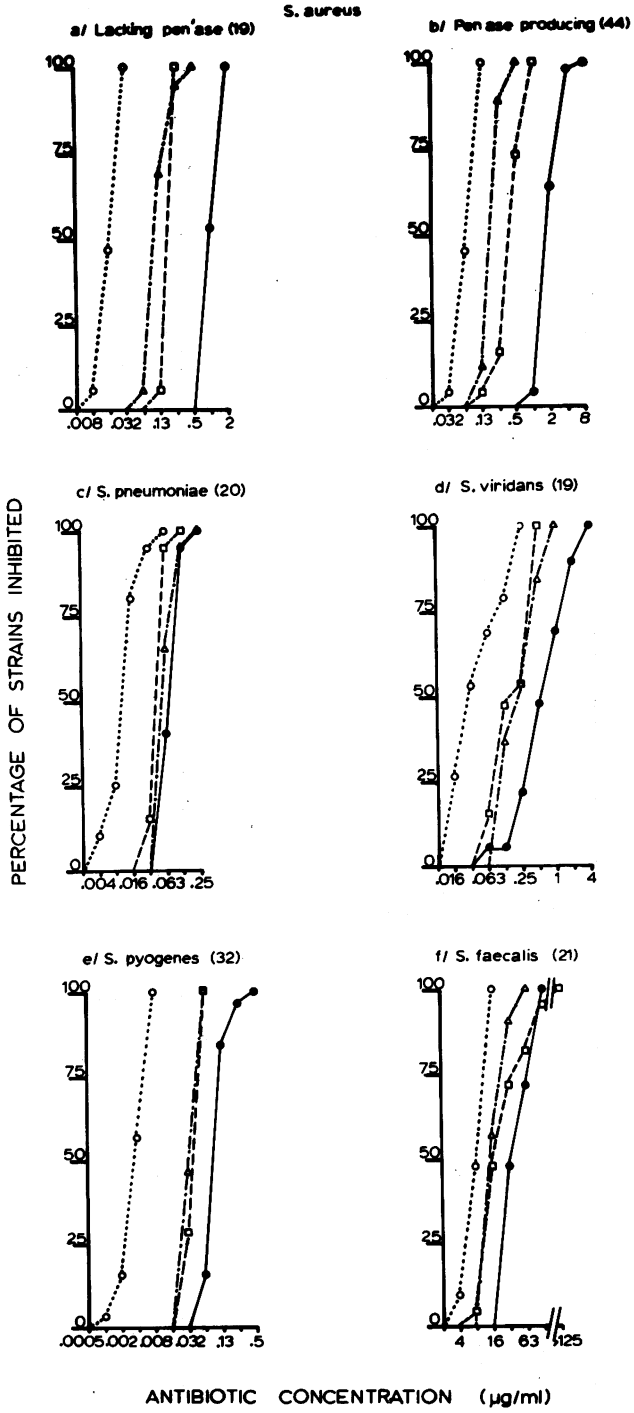

FIG. 4. *Growth-inhibitory activity of BL-S786 (●), cephalothin (△), cephaloridine (○), and cefazolin (□) against strains of various gram-positive organisms. The numeral in parentheses indicates the number of strains.*

TABLE 1. *Growth-inhibitory activity against strains of miscellaneous gram-negative organisms*

Organism	No. of strains	Minimum inhibitory concn[a] (μg/ml)			
		BL-S786	Cephalothin	Cephaloridine	Cefazolin
Edwardsiella tarda	2	0.13	1	1	0.71
Arizona hinshawii	2	0.13	2.8	2	1
Citrobacter sp.	4	1	63	>125	75
Citrobacter sp.	1	125	>125	>125	>125
Serratia marcescens	15	>125	>125	>125	>125
Erwinia sp.	5	0.66	5.3	2	1.5
Vibrio cholerae	1	4	1	16	4
Pasteurella multocida	2	0.25	0.09	0.5	0.5
Pseudomonas aeruginosa	16	>125	>125	>125	>125
Alcaligenes sp.	5	2.6	1.3	6.1	4.6
Alcaligenes sp.	2	63	>125	>125	>125
Alcaligenes sp.	4	>125	>105	>125	>105
Bordetella bronchiseptica	1	125	8	32	125
Bacteroides fragilis	3	100	125	79	50

[a] Geometric mean when applicable.

TABLE 2. *Growth-inhibitory activity against strains of miscellaneous gram-positive organisms*

Organism	No. of strains	Minimum inhibitory concn[a] (μg/ml)			
		BL-S786	Cephalothin	Cephaloridine	Cefazolin
Listeria monocytogenes	7	10.8	2	0.91	1.2
Clostridium sp.	3	2	0.5	0.63	0.4
Mycobacterium tuberculosis[b]	2	4	63	16	125
Mycobacterium tuberculosis[c]	1	16	250	63	250

[a] Geometric mean when applicable.
[b] Strain H37Rv: parent and streptomycin-resistant mutant.
[c] Strain H37Rv: isoniazid-resistant mutant.

moniae at 0.25 μg/ml, all strains of *S. pyogenes* at 0.5 μg/ml, and all strains of *S. viridans* at 4 μg/ml. The three strains of *Clostridium* sp. were inhibited at 2 μg/ml. BL-S786 was 4 to 32 times more active than the control compounds against *M. tuberculosis* H37Rv. *S. faecalis* was resistant to BL-S786.

(ii) Effect of inoculum size. A variation in the initial cell concentration of *Enterobacteriaceae* affected in a similar fashion the growth-inhibitory activity of BL-S786 and of the control compounds (Table 3). Thus, with few exceptions, the activity of all compounds declined sharply (from 16- to more than 1,000-fold) against strains of *Enterobacter cloacae, E. aerogenes,* and *P. rettgeri* but only moderately (from 2- to 8-fold) against strains of *E. coli, K. pneumoniae, P. mirabilis,* and *P. vulgaris* when the inoculum was increased from 10^2 to 10^6 organisms.

The effect of inoculum on the susceptibility of staphylococci to cephalosporins is illustrated in Table 4. The susceptibility of strains that lack penicillinase varied little with initial cell concentration. With penicillinase-producing strains, cell concentration affected only slightly the growth-inhibitory activity of BL-S786 and cephalothin, moderately that of cefazolin, and sharply that of cephaloridine.

(iii) Bactericidal activity. BL-S786 and the control compounds were effective bactericidal agents for strains of *E. coli, K. pneumoniae, P. mirabilis,* and *P. vulgaris* (Table 5). For most strains of *Enterobacter,* BL-S786 alone was significantly bactericidal. One strain of *P. rettgeri* was susceptible and the other was largely resistant to the bactericidal action of the cephalosporins.

(iv) Susceptibility to β-lactamase. Susceptibility to enzymatic hydrolysis was assessed by determining relative rates of hydrolysis by cell-free preparations of various β-lactamases (Table 6). BL-S786 was slightly to markedly less susceptible than the other cephalosporins to hydrolysis by β-lactamases of types Ia, Ib, IIIa, and IVa. All cephalosporins were poor substrates for type IIa and staphylococcal β-lactamases. The four cephalosporins were hydrolyzed at similar rates by the type IVb enzyme.

(v) Binding to serum proteins. In 95% human serum, BL-S786 and cephalothin were 67%, cephaloridine was 30%, and cefazolin was 76% protein bound.

(vi) Stability in solution. At pH 7.4 and 37°C, the half-life of BL-S786 and the control cephalosporins was greater than 24 h.

Properties in vivo. (i) Concentration in murine blood. Peak antibiotic concentrations of BL-S786 and the three control compounds were observed 0.25 h after intramuscular adminis-

TABLE 3. *Effect of inoculum size on the susceptibility of Enterobacteriaceae*

Organism	Strain	Minimum inhibitory concn (μg/ml)[a]							
		BL-S786		Cephalothin		Cephaloridine		Cefazolin	
		A	B	A	B	A	B	A	B
Escherichia coli	1	0.5	2	8	32	2	8	1	4
	3	0.25	1	4	16	2	4	1	2
	4	0.5	2	4	32	2	4	1	2
	5	1	2	4	32	4	8	1	2
	6	0.5	2	8	16	2	8	1	2
	7	0.25	2	1	8	2	4	1	2
	2	4	32	16	32	4	16	2	16
Klebsiella pneumoniae	1	0.5	1	2	8	2	4	1	2
	2	0.25	2	2	32	2	8	1	4
	3	0.5	4	2	16	2	16	1	4
	4	0.5	4	2	8	2	8	1	4
	5	0.5	2	2	32	2	16	1	4
	6	0.25	4	1	16	2	16	1	32
Enterobacter cloacae	1	1	>250	8	>250	4	>250	2	>250
	2	1	>250	16	>250	125	>250	32	>250
	3	2	>250	16	250	16	250	8	250
	4	2	>250	32	>250	>250	>250	63	>250
	5	8	250	16	250	63	>250	8	>250
Enterobacter aerogenes	1	1	>250	32	>250	63	>250	16	>250
Proteus mirabilis	1	0.5	2	8	16	4	16	4	8
	2	0.5	4	4	32	8	16	4	16
	3	0.5	2	16	32	8	32	8	32
	4	0.5	2	8	32	4	32	4	32
	5	0.5	4	8	32	4	16	4	16
Proteus vulgaris	1	1	4	1	4	4	16	4	250
	4	1	8	16	16	8	16	8	32
	2	1	8	8	125	8	250	8	32
Proteus rettgeri	1	0.13	125	0.25	250	4	250	0.5	>250
	2	0.13	63	0.25	>250	0.25	>250	0.25	>250
	3	0.25	>250	32	>250	16	>250	2	>250

[a] A, Inoculum of 10^2 organisms; B, inoculum of 10^6 organisms.

TABLE 4. *Effect of inoculum size on the susceptibility of S. aureus*

Organism type	No. of strains	MIC (μg/ml)[a]								MIC_B/MIC_A[b]			
		BL-S786		Cephalothin		Cephaloridine		Cefazolin		BL-S-786	Cephalothin	Cephaloridine	Cefazolin
		A	B	A	B	A	B	A	B				
Lacking penicillinase	3	1.6	2.5	0.13	0.13	0.025	0.039	0.13	0.20	1.6	1.0	1.6	1.6
Penicillinase producer	26	3.8	5.7	0.20	0.44	0.077	1.6	0.22	0.79	1.5	2.2	20	3.6

[a] A, Inoculum of 10^2 organisms; B, inoculum of 10^6 organisms. The values are geometric means.
[b] MIC_A, Minimum inhibitory concentration with an inoculum of 10^2 organisms; MIC_B, minimum inhibitory concentration with an inoculum of 10^6 organisms.

tration. This is illustrated in Fig. 5 (left panel) for a dose of 20 mg/kg. At this dose, BL-S786 reached a peak concentration of 36 μg/ml, compared to 31 μg/ml for cefazolin, 23 μg/ml for cephaloridine, and 17 μg/ml for cephalothin. The concentration of BL-S786 in the blood declined at a slower rate ($t_{1/2}$ of 33 min) than that of the control cephalosporins ($t_{1/2}$ of 11 min for cephalothin, 22 min for cephaloridine, and 25 min for cefazolin). With all compounds, peak concentrations were essentially proportional to dose in the range tested (Fig. 5, right panel).

(ii) Recovery in rat urine. Rats, receiving intramuscularly 10 mg of BL-S786 per kg of body weight, excreted an average of 65% of the administered dose in urine. Comparable frac-

tions of the dose were recovered when cephaloridine or cefazolin was given (59 and 75% of the dose, respectively). By contrast, only 22% of the cephalothin dose was accounted for in the urine. With all cephalosporins, over 96% of the recoverable dose was excreted within 6 h of administration.

(iii) Treatment of systemically infected mice. Against infections with *E. coli* and *K. pneumoniae* (Table 7), BL-S786, given intramuscularly, was either more active than the control cephalosporins (*E. coli* 1, *K. pneumoniae* 1) or at least as active as the most active of these compounds (*E. coli* 8 and 9, *K. pneumoniae* 7). In the treatment of infections with *P. mirabilis, P. vulgaris, P. morganii, P. rettgeri, Citrobacter* sp., and *P. stuartii,* BL-S786 was more active than the control cephalosporins. Against infections with *E. cloacae,* BL-S786 alone was effective.

In the treatment of streptococcal infections, BL-S786 was generally more active than cepha-

TABLE 5. *Minimum growth-inhibitory and bactericidal concentrations for Enterobacteriaceae*[a]

Organism	Strain	BL-S786		Cephalothin		Cephaloridine		Cefazolin	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	1	0.5	0.5	8	16	4	4	2	2
	3	0.25	0.5	4	8	2	2	2	2
	4	0.5	0.5	8	16	4	4	1	2
	5	1	1	16	16	4	4	2	2
	6	1	1	16	16	4	8	2	4
	2	16	16	32	32	16	16	8	16
Klebsiella pneumoniae	1	0.25	0.25	2	2	2	4	2	2
	2	0.25	0.25	4	8	4	4	1	2
	3	0.25	0.25	2	4	2	2	1	2
	4	0.5	0.5	4	4	4	4	2	4
	5	0.5	0.5	8	8	8	8	2	2
	6	0.5	1	2	2	2	2	2	4
Enterobacter cloacae	3	4	8	32	63	16	16	4	8
	4	8	63	250	>250	>250	>250	250	>250
	8	63	63	>250	>250	>250	>250	>250	>250
Enterobacter aerogenes	1	2	2	125	250	>250	>250	32	125
	2	2	2	125	>250	250	>250	63	>250
	3	8	16	250	>250	250	250	63	250
Proteus mirabilis	1	0.5	1	8	32	8	16	4	8
	2	0.5	0.5	8	32	8	8	8	16
	3	0.5	0.5	8	8	8	8	8	8
	4	0.25	0.5	4	8	8	8	4	8
	5	0.5	0.5	8	16	4	8	8	8
Proteus vulgaris	1	0.5	1	1	2	8	8	4	4
	4	0.5	1	8	16	8	16	8	16
	2	0.5	0.5	8	16	8	16	8	16
Proteus rettgeri	2	0.063	0.13	1	2	0.5	0.5	0.25	0.5
	3	0.25	63	125	250	32	>250	4	>250

[a] MIC, Minimum growth-inhibitory concentration; MBC, minimum bactericidal concentration; expressed as micrograms per milliliter. The inoculum was 10^4 cells.

TABLE 6. *Relative susceptibility to hydrolysis by β-lactamase in cell-free extracts*

Enzyme[a]		Organism	Strain	Relative rate of hydrolysis (benzylpenicillin = 100)			
Class	Type			BL-S786	Cephalothin	Cephaloridine	Cefazolin
I	a	*Enterobacter cloacae*	214	700	2,100	7,900	10,000
	b	*Escherichia coli*	719	170	480	370	760
II	a	*Proteus mirabilis*	1266	<1	<1	2	<1
III	a	*Escherichia coli*	TEM	2	9	93	12
IV	a	*Klebsiella pneumoniae*	53	5	8	100	12
	b	*Klebsiella pneumoniae*	1169	27	43	50	31
		Staphylococcus aureus	A9606	<0.5	<0.5	<0.5	<0.5

[a] Enzyme classification according to Richmond and Sykes (3).

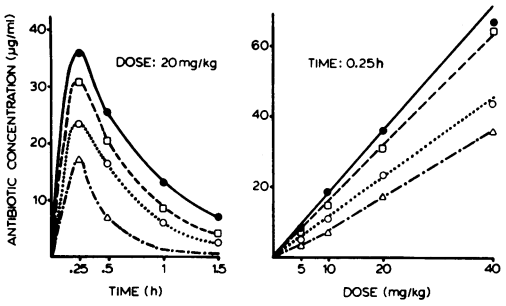

FIG. 5. *Antibiotic concentration in blood after intramuscular administration to mice. Symbols: ●, BL-S786; △, cephalothin; ○, cephaloridine; □, cefazolin.*

lothin but less active than cephaloridine and cefazolin (Table 8). Against staphylococcal infections, there was no well-defined efficacy pattern, but BL-S786 was at least as active as cephalothin.

DISCUSSION

BL-S786 compared favorably with cephalothin, cephaloridine, and cefazolin in activity and bioavailability. The compound had a broader spectrum of antibacterial activity than cephalosporins currently available for clinical use. Thus, it was active against the majority of strains of *Enterobacter* sp., *P. rettgeri*, *P.*

TABLE 7. *Efficacy of the intramuscular treatment of mice systemically infected with gram-negative organisms*[a]

Organism	Strain	Challenge (no. of organisms)	PD_{50}/treatment (mg/kg)[b]			
			BL-S786	Cephalothin	Cephaloridine	Cefazolin
Escherichia coli	1	2×10^5	0.37	42	2.6	1.9
	8	6×10^5–4×10^6	1.5	97	2.2	4.9
	9	7×10^3–2×10^4	2.2	180	13	5.3
Klebsiella pneumoniae	1	4×10^4–5×10^4	2	94	8	16
	7	9×10^2–1×10^3	22	>380	41	94
Proteus mirabilis	1	7×10^6–9×10^6	1.4	41	19	16
	5	9×10^5–3×10^6	0.62	11	4	6.2
	8	6×10^5–4×10^6	0.59	14	5.5	6.5
Proteus vulgaris	1	1×10^6–2×10^6	1.8	11	6.4	11
	3	4×10^5	2.5	17	10	8
Proteus morganii	1	1×10^5–4×10^5	19	>350	180	62
	2	2×10^6–3×10^6	39	>400	230	140
Proteus rettgeri	4	2×10^6–3×10^6	2.4	140	66	9.5
Citrobacter sp.	1	5×10^6–2×10^7	3.5	>340	36	110
Providencia stuartii	2	1×10^6–5×10^6	5.9	>380	180	50
	3	2×10^7	8	160	150	70
Enterobacter cloacae	9	2×10^5–4×10^5	1.9	>400	200	170
	10	2×10^6–3×10^6	8.6	>400	>400	>400
	7	4×10^4–3×10^5	13	>400	>400	>370

[a] Animals were treated twice: 1 and 3.5 h after infection.
[b] PD_{50}, 50% protective dose.

TABLE 8. *Efficacy of intramuscular treatment of mice systemically infected with gram-positive organisms*[a]

Organism	Strain	Challenge (no. of organisms)	PD_{50}/treatment (mg/kg)[b]			
			BL-S786	Cephalothin	Cephaloridine	Cefazolin
Streptococcus pneumoniae	1	2×10^3–9×10^3	0.52	4	0.1	0.39
	2	1×10^4–3×10^4	1.6	19	0.26	0.57
Streptococcus pyogenes	1	2×10^3–6×10^3	2.9	4.5	0.06	0.57
	2	1×10^4–5×10^4	0.93	2.7	0.02	0.27
Staphylococcus aureus	1	2×10^5–3×10^5	0.3	0.3	0.01	0.09
	2	1×10^9	5.4	9.3	3.2	5
	3	5×10^8–7×10^9	10	32	3.3	23

[a] Animals infected with penicillinase-producing staphylococcal strains (no. 2 and 3) were treated 0 and 2 h after infection; the others were treated 1 and 3.5 h after infection.
[b] PD_{50}, 50% protective dose.

stuartii, and *Citrobacter* sp., organisms typically resistant to the other cephalosporins. With few exceptions (*H. influenzae*, *N. gonorrhoeae*), BL-S786 was more active in vitro against major gram-negative pathogenic species than any of the three control cephalosporins. This advantage of BL-S786 was borne out in vivo. Although BL-S786 was generally less active than the reference compounds against gram-positive pathogens, it inhibited their growth at concentrations that should readily be achieved in humans after standard parenteral dosage. *S. faecalis*, a species relatively unsusceptible to cephalosporins in general, was an exception. BL-S786 was an effective bactericidal agent for strains of various gram-negative species, including *E. cloacae* and *E. aerogenes*. When given intramuscularly to mice, BL-S786 achieved high concentrations in blood and its biological half-life was longer than that of the other three cephalosporins. Two-thirds of the dose administered intramuscularly to rats was recovered in urine.

BL-S786 is to be subjected to clinical trials in the near future.

ACKNOWLEDGMENTS

We are indebted to the following for devoted technical assistance: M. Christine Arnold, Eileen W. Chaplinsky, Kenneth L. Den Bleyker, Kathlyn F. Frenette, Richard A. Goodhines, Thomas B. Hallett, Angela M. Hill, Thomas Ingram, Peter A Kresel, Antoinette C. Nutting, Christian E. Thater, Martha C. Thiesing, Jacqueline A. Randolph, Erwin B. Williams, and Ellen M. Yastrib. We also acknowledge the excellent clerical assistance of Evelyn Barenholtz and Maxine C. Postle.

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