

COMPARISON OF BL-S786 WITH CEPHALOTHIN, CEFAMANDOLE AND
CEFOXITIN *IN VITRO* AND IN TREATMENT OF EXPERIMENTAL
INFECTIONS IN MICE

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The activity of BL-S786 was compared to that of cephalothin, cefamandole and cefoxitin *in vitro* and in treatment of experimental infections in mice. In broth dilution tests, the activity of BL-S786 was less than cephalothin or cefamandole against *Staphylococcus aureus* and less than cefamandole or cefoxitin against *Haemophilus influenzae*. BL-S786 and cefamandole were the two most active drugs against cephalothin-sensitive Enterobacteriaceae. In tests with cephalothin-resistant Enterobacteriaceae, BL-S786 was generally less active than cefamandole but more active than cefoxitin against all strains except *Proteus* and *Providencia*. Regardless of the comparative *in vitro* activity of the four drugs, BL-S786 was the most effective drug in treatment of mice lethally infected with Enterobacteriaceae. Protection from lethality was associated with clearance of bacteremia by each of the four drugs. In several tests where *in vitro* activity was not predictive of *in vivo* efficacy, selection of resistance *in vivo* was found to have occurred.

BL-S786 is a new semisynthetic cephalosporin with a broader spectrum of activity than currently available cephalosporin antibiotics.^{1,2,3,4)} In comparative *in vitro* studies, BL-S786 was found to be more active than other cephalosporins and cefoxitin against certain Enterobacteriaceae.^{1,2,3,4)} In addition, BL-S786 has been shown to be more effective than cephalothin, cephaloridine and cefazolin in the treatment of experimental infections in mice.¹⁾ The purpose of this investigation was to compare the antimicrobial activity of BL-S786 to cephalothin, and the two expanded spectrum antibiotics, cefamandole and cefoxitin.^{5,6,7,8)} Studies included evaluation of the four drugs (1) *in vitro* against *Staphylococcus aureus*, *Haemophilus influenzae* and Enterobacteriaceae, and (2) in treatment of mice systemically infected with Enterobacteriaceae.

Methods

Bacterial Strains

All strains were recent clinical isolates and included Enterobacteriaceae, *Haemophilus influenzae* and *Staphylococcus aureus*. Enterobacteriaceae were designated cephalothin-resistant if the cephalothin zone size was 14 mm or less in disk diffusion tests; all other strains were designated cephalothin-sensitive.

Antibiotics

All drug solutions (weight compensated for purity) were prepared the day of use. For susceptibility tests, working standards were prepared from the following antibiotic powders: sodium cephalothin, cefamandole lithium (Eli Lilly and Co.), BL-S786, free acid (Bristol Laboratories), and sodium cefoxitin (Merck Sharp and Dohme). Solutions were prepared in distilled water for each drug except BL-S786 which was dissolved in phosphate buffer, pH 7.0. For mouse protection studies, solutions of the following drugs were prepared in phosphate buffer, pH 7.0: cefamandole nafate, cephalothin sodium U.S.P., sodium cefoxitin, and BL-S786, free acid.

Susceptibility tests

Serial twofold broth dilution tests were performed in a final volume of 3 ml. Tests for all antibiotics against each strain were performed simultaneously. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of drug inhibiting macroscopic growth after 18 hours incubation. Subcultures were made from each clear tube (0.01 ml, calibrated loop), and the minimal bactericidal concentration (MBC) was defined as the lowest concentration of drug preventing growth on subculture. Tests with all strains except *Haemophilus* were performed in MUELLER-HINTON broth (MHB, BBL), inoculated with $1 \sim 5 \times 10^4$ CFU/ml (final bacterial test population) from overnight MHB cultures, and incubated in air. Subcultures were made onto sheep blood agar (BAP). Tests with *Haemophilus* were performed in modified LEVINTHAL broth (LB), inoculated with $1 \sim 5 \times 10^6$ CFU/ml from overnight LB cultures, and incubated in 10% CO₂ in air. Subcultures were made onto chocolate agar.

Serial twofold agar dilution tests were performed on MUELLER-HINTON agar, inoculated with $1 \sim 5 \times 10^4$ CFU by a replicating device,⁹⁾ and incubated in air. The MIC was defined as the lowest concentration of drug preventing all growth after 18 hours incubation. Disk diffusion tests were performed by the method of BAUER *et al.*¹⁰⁾ with 30 µg disks of each drug.

Mouse Protection Tests

Challenge strains were incubated with shaking in Brain Heart Infusion broth (Difco) until late exponential phase. Cells were then harvested by centrifugation, washed and resuspended in 0.5% NaCl. These suspensions were diluted in 4% hog gastric mucin (American Laboratories, Omaha, NE.) and 0.5 ml injected intraperitoneally into Swiss-Webster ICR strain mice weighing 20~25 g (Sasco, Inc., Omaha, NE.). Each antibiotic was administered intramuscularly (0.2 ml) at 1.0 and 3.5 hours post infection at five different dosages to groups of 10 mice each. The dose in mg/kg required to protect 50% of the animals from death (PD₅₀) at 48 hours was calculated by use of log probit plots. The actual lethality of the challenge inoculum was determined from the number of deaths in an infected but untreated control group. A second control group consisted of mice injected with the suspending medium only.

Microbial analysis of the effect of therapy on each infection was performed on several animals from the various treatment and control groups. Cultures of heart blood and peritoneum were taken at the time of death or, for survivors, following sacrifice by cervical dislocation at 48 hours. Animals cultured included (1) controls and (2) members of the treatment groups nearest the PD₅₀ or members of the highest dose treatment group in tests where protection by the drug failed to occur. The identity of challenge strains recovered on these cultures was confirmed by standard biochemical tests (Enterotube, Roche Laboratories). Peritoneal cultures from uninfected animals, when occasionally positive, never contained organisms similar to the challenge strains and heart blood cultures were always sterile. Isolates of each challenge strain recovered from the treated animals were tested for susceptibility to BL-S786, cephalothin, cefamandole and cefoxitin by agar and broth dilution assays and compared to that of the challenge strain prior to animal passage. Peritoneal isolates were used in these tests only when heart blood cultures were sterile. Isolates were not transferred from the primary culture plate prior to testing. Results of each of these tests were used to determine if (1) protection from lethality by each of the four drugs was associated with eradication of the challenge strain and (2) failure to protect from lethality was associated with failure to eliminate the challenge strain and/or emergence of resistance to the antibiotic.

Results

Broth Dilution Susceptibility Tests

The *in vitro* activity of BL-S786, cefamandole (CM), cefoxitin (CX) and cephalothin (CF) was determined against eight strains of *Staphylococcus aureus*, 15 strains of *Haemophilus influenzae*, 40 CF-sensitive Enterobacteriaceae, and 120 CF-resistant Enterobacteriaceae. As shown in Table 1, BL-S786 was less active than CM or CF against both penicillin- and methicillin-resistant *S. aureus*. MICs

Table 1. Comparative activity of BL-S786, cefamandole, cefoxitin and cephalothin against *Staphylococcus aureus*

	Penicillin resistant (4) ^a		Methicillin resistant (4)	
	MIC	MBC	MIC	MBC
BL-S786	1.6~3.1 ^b	3.1~6.2	6.2~>100	25~>100
Cefamandole	≤0.4	≤0.4	≤0.4~0.8	1.6~3.1
Cefoxitin	1.6~3.1	3.1	12.5	12.5~50.0
Cephalothin	≤0.4	≤0.4	≤0.4~1.6	3.1~12.5

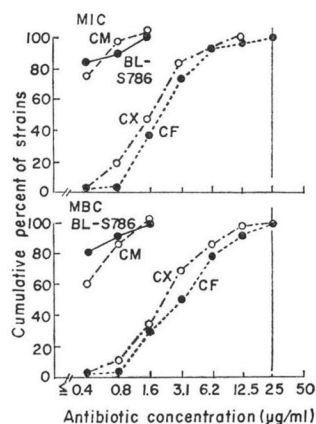
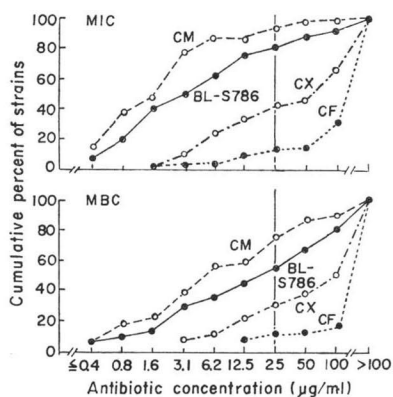
^a number of strains. ^b range in $\mu\text{g/ml}$.

Table 2. Comparative activity of BL-S786, cefamandole, cefoxitin and cephalothin against *Haemophilus influenzae*

	Ampicillin sensitive (8) ^a		Ampicillin resistant (7)	
	MIC	MBC	MIC	MBC
BL-S786	0.8~1.6 ^b	1.6~6.2	0.1~6.2	3.1~>25.0
Cefamandole	≤0.05~0.2	≤0.05~6.2	≤0.05~0.8	0.2~25.0
Cefoxitin	0.8~1.6	0.8~3.1	≤0.05~1.6	0.2~6.2
Cephalothin	0.4~0.8	0.8~3.1	≤0.05~0.8	1.6~>25.0

^a number of strains ^b range in $\mu\text{g/ml}$

Fig. 1. Comparative activity of BL-S786, cefamandole, cefoxitin and cephalothin against 40 cephalothin-sensitive Enterobacteriaceae.

Fig. 2. Comparative activity of BL-S786, cefamandole, cefoxitin and cephalothin against 42 cephalothin-resistant *Enterobacter* sp.

and MBCs of CM and CF were four to 16-fold lower than those of BL-S786 or CX against penicillin-resistant strains and four to over 256-fold lower against methicillin-resistant strains. In bactericidal tests with *H. influenzae*, the activity of BL-S786 was similar to CF, less than CM, and less than CX against ampicillin-resistant strains (Table 2). In tests with 40 CF-sensitive Enterobacteriaceae (included *E. coli*, *Klebsiella*, *Citrobacter*, *Salmonella* and *P. mirabilis*), BL-S786 and CM were the most active agents with MICs and MBCs of 1.6 $\mu\text{g/ml}$ or less for all strains (Fig. 1). At 1.6 $\mu\text{g/ml}$, less than one-half of these strains were inhibited or killed by CX or CF. The relative order of activity of the four drugs did not vary when results were compared for each of the genera included in these 40 strains.

Fig. 3. Comparative activity of BL-S786, cefamandole, cefoxitin and cephalothin against 50 cephalothin-resistant indole-positive *Proteus* and *Providencia*.

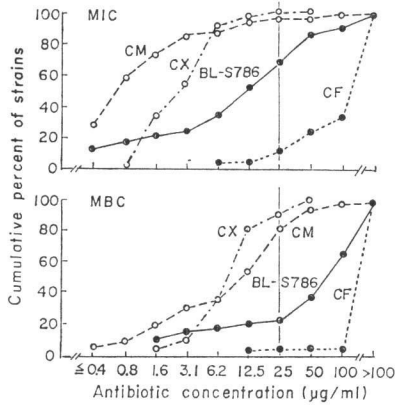
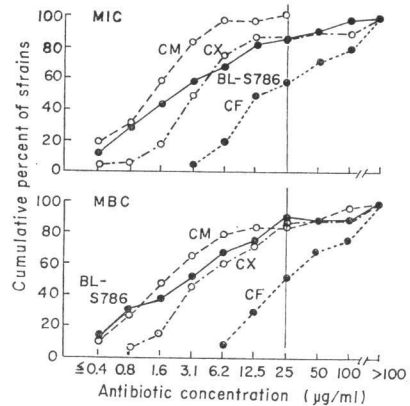


Fig. 4. Comparative activity of BL-S786, cefamandole, cefoxitin and cephalothin against 28 other cephalothin-resistant Enterobacteriaceae. Includes six *Klebsiella*, 17 *E. coli*, four *Serratia*, and one *Citrobacter*.



Against 120 CF-resistant Enterobacteriaceae, the relative activity of the four antibiotics did vary with the genus of organism tested. In tests with 42 *Enterobacter* sp., the activity of BL-S786 was slightly less than CM and greater than either CX or CF (Fig. 2). Against 50 indole-positive *Proteus* and *Providencia*, BL-S786 was less active than CM or CX but more active than CF (Fig. 3). The relative order of activity of the four drugs did not vary when results were compared for each species included among the *Enterobacter*, indole-positive *Proteus* and *Providencia*. Against 28 other CF-resistant Enterobacteriaceae (included six *Klebsiella*, 17 *E. coli*, four *Serratia* and one *Citrobacter*), the activity of BL-S786, CM and CX was similar (Fig. 4). At 25 µg/ml or less, over 80% of strains were inhibited or killed by each of the three agents. Among these four genera, the *Serratia* were least susceptible to the three agents (Tables 3 and 4).

Table 3. Comparative MICs of BL-S786 (BLS), cephalothin (CF), cefamandole (CM), and cefoxitin (CX) against 28 miscellaneous cephalothin-resistant Enterobacteriaceae

Organism	No. strains	Drug	Drug concentrations (µg/ml) and cumulative percent of strains inhibited									
			≤0.4	0.8	1.6	3.1	6.2	12.5	25	50	100	>100
<i>Klebsiella</i> sp.	6	BLS	33	33	67	67	67	83	100	—	—	—
		CF	—	—	—	17	33	50	50	50	67	100
		CM	17	17	83	100	—	—	—	—	—	—
		CX	—	—	33	50	67	67	67	67	67	100
<i>E. coli</i>	17	BLS	6	35	47	71	82	94	100	—	—	—
		CF	—	—	—	—	18	65	77	94	100	—
		CM	18	35	59	71	94	94	100	—	—	—
		CX	6	6	18	65	82	94	94	94	94	100
<i>Serratia</i> sp.	4	BLS	—	—	—	—	—	25	25	25	75	100
		CF	—	—	—	—	—	—	—	—	—	100
		CM	—	—	—	100	—	—	—	—	—	—
		CX	—	—	—	—	75	75	75	100	—	—
<i>Citrobacter</i> sp.	1	BLS	—	—	—	—	100	—	—	—	—	—
		CF	—	—	—	—	—	—	—	100	—	—
		CM	100	—	—	—	—	—	—	—	—	—
		CX	—	—	—	—	—	100	—	—	—	—

Table 4. Comparative MBCs of BL-S786 (BLS), cephalothin (CF), cefamandole (CM) and cefoxitin (CX) against 28 miscellaneous cephalothin-resistant Enterobacteriaceae

Organism	No. strains	Drug	Drug concentrations ($\mu\text{g/ml}$) and cumulative percent of strains killed									
			≤ 0.4	0.8	1.6	3.1	6.2	12.5	25	50	100	> 100
<i>Klebsiella</i> sp.	6	BLS	33	33	33	33	50	67	100	—	—	—
		CF	—	—	—	—	17	50	50	50	100	
		CM	—	17	67	67	67	83	83	100	—	—
		CX	—	—	33	50	67	67	67	67	67	100
<i>E. coli</i>	17	BLS	6	35	47	71	82	94	100	—	—	—
		CF	—	—	—	—	6	29	65	94	100	—
		CM	6	29	47	71	88	88	88	94	100	—
		CX	—	6	12	59	82	88	94	94	94	100
<i>Serratia</i> sp.	4	BLS	—	—	—	—	—	—	25	25	25	100
		CF	—	—	—	—	—	—	—	—	—	100
		CM	—	—	—	25	50	50	50	50	75	100
		CX	—	—	—	—	—	25	75	100	—	—
<i>Citrobacter</i> sp.	1	BLS	—	—	—	—	100	—	—	—	—	—
		CF	—	—	—	—	—	—	—	—	100	—
		CM	100	—	—	—	—	—	—	—	—	—
		CX	—	—	—	—	—	—	100	—	—	—

Disk Diffusion Tests

Results of disk diffusion tests with 160 Enterobacteriaceae are shown in Table 5. Mean zone sizes of CM were generally larger than BL-S786 and mean zone sizes of both were larger than CX. Although zone sizes of each of the three drugs were larger in tests with strains inhibited or killed by $\leq 25 \mu\text{g/ml}$ in dilution assays, wide ranges in zone sizes did occur. When zone sizes were compared with MICs, all strains resistant to inhibition by $25 \mu\text{g/ml}$ CM, BL-S786 or CX had zone sizes less than 22 mm, 18 mm and 14 mm respectively. Results varied somewhat when comparisons were based on MBCs. Strains could not be separated into two distinct groups based on MBCs and zone sizes of either BL-S786 or CM. For CX, all strains not killed by $25 \mu\text{g/ml}$ had zone sizes less than 14 mm.

Table 5. Comparison of results in disk diffusion and broth dilution tests with BL-S786, cefamandole and cefoxitin against 160 Enterobacteriaceae

Broth dilution results		No. strains	Disk ^a diffusion results (mm) and percent of strains with zone					
			Mean (range)	≥ 22	≥ 20	≥ 18	≥ 16	> 14
MIC $\leq 25 \mu\text{g/ml}$	BL-S786	135	22 (6~31)	60	70	79	82	87
	Cefamandole	154	25 (6~35)	82	84	86	87	91
	Cefoxitin	132	18 (6~28)	43	59	61	70	81
MIC $> 25 \mu\text{g/ml}$	BL-S786	25	8 (6~17)	0	0	0	4	8
	Cefamandole	6	15 (6~21)	0	17	33	50	67
	Cefoxitin	28	6 (6~9)	0	0	0	0	0
MBC $\leq 25 \mu\text{g/ml}$	BL-S786	99	25 (12~31)	73	85	95	97	98
	Cefamandole	136	24 (6~35)	83	90	91	92	93
	Cefoxitin	124	19 (6~28)	47	63	65	76	87
MBC $> 25 \mu\text{g/ml}$	BL-S786	61	12 (6~28)	16	16	18	26	33
	Cefamandole	24	19 (6~30)	33	42	46	54	64
	Cefoxitin	36	7 (6~12)	0	0	0	0	0

^a 30 μg disks

Mouse Protection Studies

Six Enterobacteriaceae of varying susceptibility to BL-S786, CM, CX and CF were selected for use as challenge strains (Table 6). Results of three different susceptibility tests indicated one strain was highly sensitive to all four drugs (*K. pneumoniae* 36); one strain was resistant only to CF (*Prov. stuartii* 36); one strain was resistant to CX and CF, but sensitive to BL-S786 and CM (*E. aerogenes* 30); and one strain was resistant to CX and CF, sensitive to CM, and moderately sensitive to BL-S786 (*E. cloacae* 55). The last two strains were chosen for study because discrepancies between results of the three *in vitro* tests gave a variable susceptibility pattern, *i.e.* sensitive in some tests, resistant in others. One strain was sensitive to CM and CX, variable to BL-S786 and resistant to CF (*P. rettgeri* 123), the other was resistant to CX and CF and variable to BL-S786 and CM (*E. cloacae* 3221).

To determine the cause of death in the infection model used in protection tests, results of heart blood and peritoneal cultures were analyzed. Since results were the same for each of the six strains, the data were pooled for this analysis (Table 7). Among animals cultured in the infected, untreated control group, the percentage with heart blood cultures positive for the challenge strain was significantly higher among animals dying from infection within 48 hours (100%) than among survivors (43%, chi-square, YATES correction, $p < 0.05$). Although a higher percentage of animals dying from infection had peritoneal cultures positive for the challenge strain, the difference between dead animals and survivors was not significant. Thus these results indicated that in the infection model studied, death was associated with bacteremia. A similar analysis was performed on results of cultures taken from animals in the various drug treated groups (Table 7). The same differences were observed (*i.e.* a

Table 6. Results of *in vitro* susceptibility tests with six Enterobacteriaceae used as challenge strains in mouse protection tests

Strain		BL-S786	CM	CX	CF
<i>K. pneumoniae</i> 36	Broth MIC/MBC ^a	0.2/0.4	0.1/0.2	1.6/1.6	0.8/0.8
	Agar MIC ^a	0.1	0.2	0.8	0.4
	Zone with 30 µg disk	29 mm	30 mm	24 mm	29 mm
<i>Prov. stuartii</i> 36	Broth MIC/MBC	3.1/12.5	0.8/0.8	1.6/3.1	100/ >400
	Agar MIC	1.6	0.4	0.4	25
	Zone with 30 µg disk	29 mm	31 mm	23 mm	9 mm
<i>P. rettgeri</i> 123	Broth MIC/MBC	0.8/50	0.8/0.8	1.6/6.2	25/50
	Agar MIC	0.2	0.4	1.6	12.5
	Zone with 30 µg disk	28 mm	29 mm	24 mm	6 mm
<i>E. aerogenes</i> 30	Broth MIC/MBC	0.8/1.6	0.8/0.8	100/200	25/400
	Agar MIC	0.8	0.4	100	6.2
	Zone with 30 µg disk	20 mm	24 mm	6 mm	6 mm
<i>E. cloacae</i> 55	Broth MIC/MBC	6.2/25	1.6/1.6	50/100	>400/ >400
	Agar MIC	6.2	1.6	100	200
	Zone with 30 µg disk	18 mm	24 mm	6 mm	6 mm
<i>E. cloacae</i> 3221	Broth MIC/MBC	6.2/100	3.1/100	100/100	>400/ >400
	Agar MIC	6.2	3.1	100	400
	Zone with 30 µg disk	17 mm	22 mm	6 mm	6 mm

^a results in µg/ml

Table 7. Microbial analysis of the infection model used in protection tests

	Recovery of challenge strain on cultures from					
	Survivors sacrificed at 48 hours			Animals dying within 48 hours		
	No. cultured	Heart blood	Peritoneum	No. cultured	Heart blood	Peritoneum
None	7	3 (43%) ^c	5 (71%)	11	11 (100%)	11 (100%)
BL-S786	32	15 (47%) ^{b,c}	27 (84%)	18	18 (100%)	18 (100%)
Cefamandole	32	14 (44%) ^c	25 (78%)	20	19 (95%)	20 (100%)
Cefoxitin	34	21 (62%) ^c	33 (97%)	27	27 (100%)	27 (100%)
Cephalothin	31	19 (61%) ^c	26 (84%)	24	24 (100%)	24 (100%)

^a infected intraperitoneally, treated at one and 3.5 hours post infection.

^b number (percent) of animals with culture positive for challenge strain.

^c significantly lower than respective value for animals dying of infection, $p < 0.05$.

Table 8. Comparison of BL-S786, cefamandole, cefoxitin and cephalothin in treatment of mice infected with Enterobacteriaceae

	Susceptibility pattern ^a	Challenge (CFU)	Inoculum (\times LD ₅₀)	PD ₅₀ (mg/kg) ^b			
				BL-S786	CM	CX	CF
<i>K. pneumoniae</i> 36	S/S/S/S	3.7×10^7	28	7.2 (4.3~10.1) ^c	10.5 (7.1~13.9)	12.0 (7.5~16.5)	19.0 (14.1~23.9)
<i>Prov. stuartii</i> 36	S/S/S/R	8.0×10^6	6	4.2 (2.9~5.5)	6.0 (1.8~10.2)	12.0 (1.0~25.0)	54.0 (30.5~77.5)
<i>P. rettgeri</i> 123	V/S/S/R	4.2×10^7	44	6.8 (1.0~15.1)	10.0 (1.7~18.3)	15.0 (11.0~19.0)	40.0 (29.6~50.4)
<i>E. aerogenes</i> 30	S/S/R/R	1.3×10^7	300	85 (69.2~100.8)	>100	>200	>200
<i>E. cloacae</i> 55	I/S/R/R	1.3×10^5	9	<4.0	25 (3.4~46.6)	58 (44.6~71.4)	>200
<i>E. cloacae</i> 3221	V/V/R/R	1.0×10^7	300	>200	>200	>200	>200

^a from Table 3, listed for BL-S786/CM/CX/CF, S=sensitive, R=resistant, V=variable, I=moderately sensitive.

^b dose required to protect 50% of animals from death at 48 hours, administered one and 3.5 hours post infection.

^c 95% confidence limit.

significantly higher percentage of animals dying from infection had bacteremia than survivors) suggesting that failure of any of the four drugs to protect an animal from death was associated with failure to clear the bacteremia.

The dose of each of the four drugs required to protect 50% of mice lethally infected with each strain is shown in Table 8. Neither the susceptibility pattern nor the comparative *in vitro* activity of the four drugs (Table 3) was predictive of the observed relative *in vivo* efficacy. In five of the six protection tests, BL-S786 gave the lowest PD₅₀, followed by CM, CX and CF respectively. In three of these tests (*Prov. stuartii* 36, *P. rettgeri* 123, and *E. cloacae* 55), PD₅₀'s were lower for BL-S786 than CM even though BL-S786 was four to 64-fold less active *in vitro*. In one test (*E. aerogenes* 30), 100 mg/kg CM was not protective even though the challenge strain was killed by 0.8 μ g/ml *in vitro*. PD₅₀'s of BL-S786 were two to 15-fold lower than CX and two to 50-fold lower than CF in tests with five of the six strains. None of the four drugs was protective at 200 mg/kg for mice infected with *E. cloacae*

3221 even though MICs of BL-S786 and CM were 6.2 and 3.1 $\mu\text{g/ml}$ respectively.

Since the *in vitro* activity of each of the four drugs was not always predictive of *in vivo* efficacy, the susceptibility of isolates recovered from infected animals to the four drugs was tested to determine if *in vivo* selection of resistance had occurred. An animal isolate was considered to be more resistant than the original challenge strain if the MIC of the drug used in therapy was increased four-fold or more. The overall occurrence of more resistant isolates in the six protection tests is shown for each drug in Table 9. Selection of resistant isolates occurred more frequently in animals treated with BL-S786 than with any of the other three drugs. Each of these isolates was also more resistant to CM. Nineteen percent of isolates tested from CM or CF treated animals were more resistant to the drug used in therapy, and each was also more resistant to BL-S786. Selection of more resistant isolates by CX occurred infrequently, and resistant isolates selected *in vivo* by the other three drugs were rarely more resistant to CX. No changes in the susceptibility of isolates from untreated control animals to the four drugs were observed.

Although *in vivo* selection of more resistant isolates was detected with each of the four drugs, in only a few instances did this appear to have affected the results of protection tests. Results of agar dilution tests with these isolates is shown in Table 10. Results of broth dilution tests were

Table 9. *In vivo* selection of resistance by BL-S786, cefamandole, cefoxitin or cephalothin

	Isolates from animals treated with			
	BL-S786	CM	CX	CF
No. tested	42	36	46	32
No. (%)				
1. more resistant ^a to drug used in therapy	12 (29%)	7 (19%)	2 (4%)	6 (19%)
2. more resistant to BL-S786	—	7	2	6
CM	12	—	2	4
CX	1	0	—	1
CF	7	1	1	—

^a MIC animals isolate/MIC challenge strain ≥ 4

Table 10. Changes in the *in vitro* susceptibility of challenge strains recovered from dead animals that had been treated with BL-S786 or cefamandole

Isolates from animals infected with	Treatment	Agar dilution MIC ($\mu\text{g/ml}$)			
		BL-S786	CM	CX	CF
<i>E. cloacae</i> 55	none ^a	6.2	1.6	100	200
	CM (16~100) ^b	25~>400 ^c	25~200	100	>400
<i>E. aerogenes</i> 30	none	0.8	0.4	200	6.2
	BL-S786 (16~100)	50~100	50~100	200	>400
<i>E. cloacae</i> 3221	none	6.2	3.1	100	400
	BL-S786 (200)	>400	>400	100	>400
	CM (200)	>400	>400	100	>400

^a isolates from untreated controls

^b dose in mg/kg

^c range observed with isolates from the different dosage groups

similar and thus are not shown. In tests with mice infected with *E. cloacae* 55, isolates more resistant to CM were recovered from dead animals that had been treated with 16~100 mg/kg CM. Such isolates were not recovered from BL-S786 treated animals. Isolates more resistant to BL-S786 were recovered from dead animals that had been infected with *E. aerogenes* 30 and treated with 16~100 mg/kg BL-S786. Such isolates were not recovered from animals treated with CM. All isolates recovered from dead animals that had been infected with *E. cloacae* 3221 and treated with 200 mg/kg CM or BL-S786 were resistant to >400 µg/ml of each of the two drugs.

Discussion

Results of *in vitro* tests indicated that BL-S786 not only had a greater spectrum of activity than cephalothin but was also more active against cephalothin-sensitive Enterobacteriaceae. *In vitro* comparisons with cefamandole, another expanded spectrum cephalosporin,^{5,8)} and cefoxitin, a cephamycin,^{6,7)} showed BL-S786 to be generally less active than cefamandole but more active than cefoxitin against all strains except *Haemophilus*, *Proteus* and *Providencia*.

When the four drugs were evaluated in treatment of mice lethally infected with Enterobacteriaceae, BL-S786 was the most effective agent followed by cefamandole, cefoxitin and cephalothin respectively. In the infection model studied, protection (increased survival rates over that of controls) by each drug administered intramuscularly was associated with clearance of the bacteremia which developed following intraperitoneal challenge. Thus, complete interpretation of results in protection tests must be based on both the *in vitro* activity and pharmacokinetics of the four drugs. Although the pharmacokinetics of the four drugs have not been compared in a single study, the pharmacokinetics in mice of each of the three expanded spectrum antibiotics have been compared with cephalothin and cephaloridine in separate studies.^{1,6,8)} A compilation of data from these studies suggests BL-S786 would produce the highest serum levels and have the longest serum half life, followed by cefamandole, cefoxitin and cephalothin respectively. These pharmacologic differences help in part to explain the greater *in vivo* efficacy of BL-S786 despite its equivalent or lower *in vitro* activity against some strains. However, in certain protection tests, results could not be explained solely by differences between the four drugs in pharmacokinetics or *in vitro* activity.

In vivo selection of resistance appeared to influence results in the three tests with mice infected with *Enterobacter* sp. Selection of resistance by cefamandole but not BL-S786 in mice infected with *E. cloacae* 55 may have contributed to the six-fold greater *in vivo* efficacy of BL-S786 despite four-fold lower *in vitro* activity against the challenge strain. Selection of resistance by BL-S786 was probably responsible for the unusually high PD₅₀ observed in mice infected with *E. aerogenes* 30 in comparison to PD₅₀'s obtained in tests with animals infected with other strains equally or less sensitive to BL-S786 *in vitro*. However, the complete failure of cefamandole to protect mice infected with *E. aerogenes* 30 could not be explained by selection of resistance, unless the resistance was so unstable that it could be detected only by direct assay with primary cultures from the animals. The single instance of complete drug failure that appeared to be due to *in vivo* selection of resistance was the failure of BL-S786 or cefamandole to protect animals infected with *E. cloacae* 3221. MICs of the two drugs against the challenge strain were 3.1 and 6.2 µg/ml, while all isolates recovered from animals following therapy were resistant to 400 µg/ml of either drug. Although the high MBCs (100 µg/ml) of the two drugs against this challenge strain may have predicted the therapeutic failure and selection of resistance observed with this strain, such *in vitro* results were not always predictive of failure. BL-S786 was very effective in treatment of mice infected with *P. rettgeri* 123 even though its MBC (50 µg/ml) indicated resistance to the drug and was significantly higher than the MIC (0.8 µg/ml). Furthermore, as noted above, cefamandole failed to protect mice infected with *E. aerogenes* 30 even though there was no discrepancy between results of *in vitro* susceptibility tests and all results indicated sensitivity to the drug.

This lack of correlation between results of *in vitro* tests and *in vivo* efficacy warrants further

investigation. Other investigators have observed various discrepancies between results of *in vitro* tests with cefamandole,^{5,11,12,13} some of which appear to be due to *in vitro* selection of resistant mutants.¹² Although similar discrepancies were observed in this study and *in vivo* selection of resistance was demonstrated, neither correlated well with the outcome of *in vivo* tests.

It should also be noted that a significant degree of cross-resistance did occur among the resistant isolates selected *in vivo* by each drug. All resistant isolates selected *in vivo* by BL-S786 were also resistant to cefamandole and *vice versa*. Cross-resistance to cefoxitin, on the other hand, was rare among these isolates. *In vivo* selection of resistance by cefoxitin did not occur frequently; however, the few resistant isolates recovered were also resistant to cefamandole and BL-S786. Although most of the challenge strains were already resistant to cephalothin prior to the protection tests, the level of resistance increased in several isolates recovered from cephalothin-treated animals, and the majority of these were then cross-resistant to BL-S786 and cefamandole. Further investigation will be required to ascertain the potential clinical significance of the (1) *in vivo* selection of resistance, (2) high degree of cross-resistance, and (3) lack of predictability of *in vivo* efficacy from *in vitro* activity observed in this study.

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