IN VITRO ACTIVITY OF BL-S217, A NEW CEPHALOSPORIN ANTIBIOTIC

FRIEDA CASEY and GERALD P. BODEY*

Department of Developmental Therapeutics, The University of Texas System Cancer Center,
M. D. Anderson Hospital and Tumor Institute,
6723 Bertner, Houston, Texas 77025, U.S.A.

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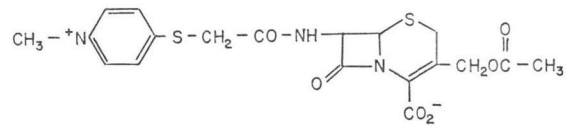
BL-S217 is a new cephalosporin derivative which is active *in vitro* against gram-positive cocci and gram-negative bacilli. At a concentration of 12.5 μg/ml, BL-S217 inhibited 83% of isolates of *Klebsiella* spp., 77% of isolates of *Escherichia coli* and 67% of isolates of *Proteus mirabilis*. BL-S217 was as active *in vitro* as most other cephalosporin antibiotics.

Gram-negative bacilli are becoming an increasingly common cause of serious infection in hospitalized patients. The discovery of the cephalosporin antibiotics was an important advance because of their activity against some gram-negative bacilli, including *Escherichia coli*, *Klebsiella* spp. and *Proteus mirabilis*. These drugs also are active against many gram-positive cocci, including penicillin G resistant *Staphylococcus aureus*. Since the introduction of cephalothin, several new derivatives have been synthesized which offer the important advantages of oral administration, less irritation following parenteral administration and higher and more prolonged serum concentrations[1,2]. Recently, another cephalosporin derivative, 7-[α-(1-methyl-4-pyridiniothio)-acetamido] cephalosporanic acid (BL-S217) has been synthesized (Fig. 1). Initial *in vitro* studies of this antibiotic indicated that it was more active than cephalothin against some gram-negative bacilli[3]. Consequently, the present study was initiated to evaluate the *in vitro* activity of BL-S217 against clinical isolates of bacteria and to compare the activity of this antibiotic with other cephalosporins.

Fig. 1. Chemical structure of BL-S217.



7-[α-(1-Methyl-4-pyridiniothio)-acetamido] cephalosporanic acid

Materials and Methods

The susceptibility testing was performed on 425 clinical isolates of gram-positive cocci and gram-negative bacilli using a microdilutor technique[4]. The organisms were inoculated into MUELLER HINTON broth, with 2% human blood added for *Streptococcus pyogenes* and *Diplococcus pneumoniae*, and incubated for 18 hours at 37°C. A 10^{-3} dilution of the broth cultures was made using MUELLER-HINTON broth and a 50 μl sample was used as the inoculum for susceptibility testing. Because the growth of *S. pyogenes* was not as great as other organisms, a 10^{-2} dilution was used.

The antibiotics, cephalexin, cephaloridine and cephalothin, were supplied by Eli Lilly and

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* Dr. BODEY is a Scholar of The Leukemia Society of America, Inc.

Co., Indianapolis, Indiana. Cephapirin and the new cephalosporin, BL-S217, were supplied by Bristol Laboratories, Syracuse, New York. All the antibiotics were diluted in MUELLER-HINTON broth to an initial concentration of 400 μg/ml. Twofold dilutions were made with broth, using 50 μl samples. After incubation for 18 hours at 37°C, the minimum inhibitory concentration (MIC) was determined. All studies were performed in triplicate.

All organisms were isolated from patients between December 1966 and January 1973. Most of the patients had underlying malignant diseases. A total of 50 isolates of *Staphylococcus aureus*, 15 isolates of *S. pyogenes*, 13 isolates of *D. pneumoniae*, 100 isolates of *E. coli*, 100 isolates of *Klebsiella* spp., 62 isolates of *Enterobacter* spp., 60 isolates of *Proteus* spp., 24 isolates of *Serratia marcescens*, and 25 isolates of *Pseudomonas aeruginosa* were tested.

Results

BL-S217 was more effective against gram-positive cocci than against gram-negative bacilli (Fig. 2). One hundred percent of both those *S. aureus* sensitive to 0.1 μg/ml of penicillin G and all of those isolates resistant to 50 μg/ml of penicillin G were inhibited by 0.78 μg/ml of BL-S217. All strains of *D. pneumoniae* were inhibited by 0.006 μg/ml. A concentration of

Fig. 2. *In vitro* activity of BL-S217 against gram-positive cocci and gram-negative bacilli.

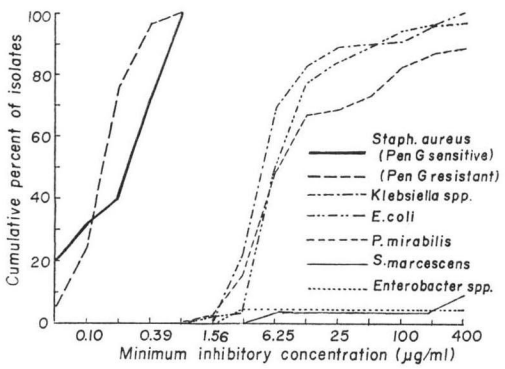


Fig. 3. Effect of inoculum size on the activity of BL-S217
a) against *Escherichia coli*. Ten isolates were tested.
b) against *Klebsiella* spp. Ten isolates were tested.

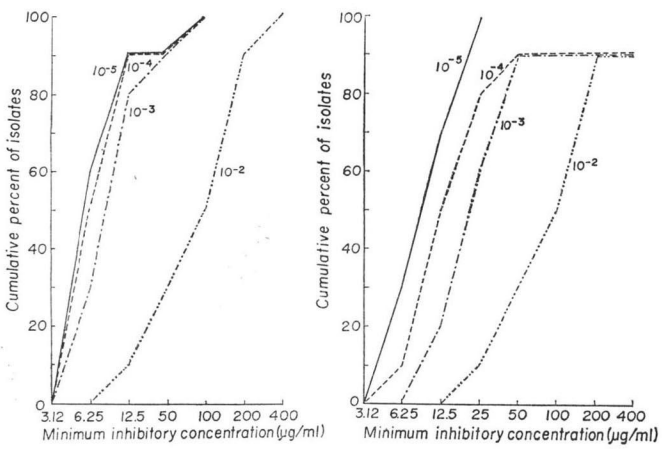


Fig. 4. Activity of cephalosporins against penicillin G sensitive *Staphylococcus aureus*. Twenty-five isolates were tested.

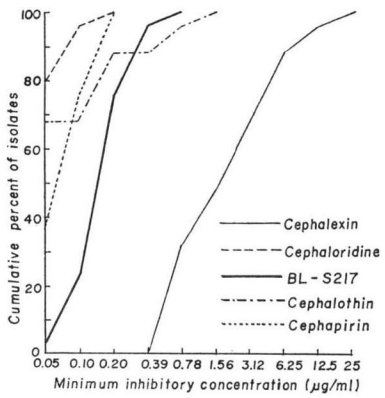
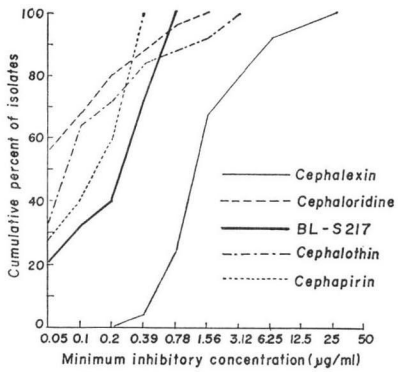


Fig. 5. Activity of cephalosporins against penicillin G resistant *Staphylococcus aureus*. Twenty-five isolates were tested.



0.05 μg/ml of BL-S217 inhibited all of the isolates of *S. pyogenes*, and 67% were inhibited by 0.0006 μg/ml.

Of the gram-negative bacilli tested, BL-S217 was most effective against *Klebsiella* spp., inhibiting 83% at a concentration of 12.5 μg/ml. This concentration also inhibited 77% of isolates of *E. coli* and 67% of isolates of *P. mirabilis*. BL-S217 was inactive against *P. aeruginosa*

Fig. 6. Activity of cephalosporins against *Streptococcus pyogenes*. Fifteen isolates were tested.

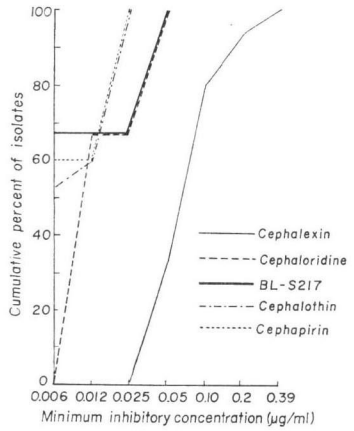


Fig. 7. Activity of cephalosporins against *Klebsiella* spp. One hundred isolates were tested.

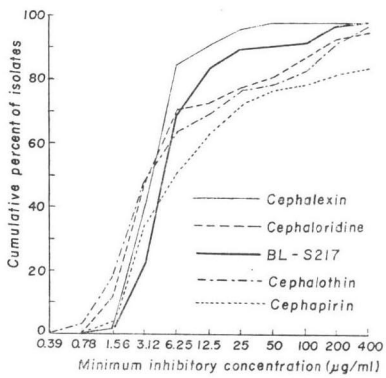


Fig. 8. Activity of cephalosporins against *Escherichia coli*. One hundred isolates were tested.

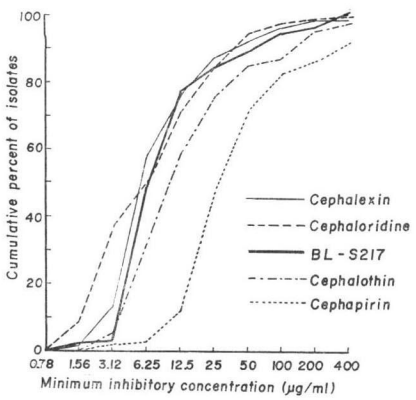


Fig. 9. Activity of cephalosporins against *Proteus mirabilis*. Forty-five isolates were tested.

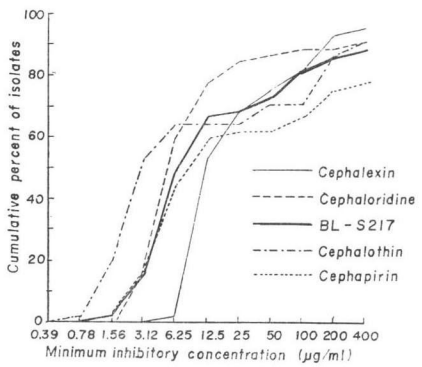


Table 1. Sensitivity of cephalosporin resistant organisms to BL-S217

	P. mirabilis		*E. coli*		*Klebsiella* sp.	
Cephalosporin	Number resistant to other cephalosporin	Number sensitive to BL-S217	Number resistant to other cephalosporin	Number sensitive to BL-S217	Number resistant to other cephalosporin	Number sensitive to BL-S217
Cephalexin	14	1	13	0	5	0
Cephaloridine	7	0	16	3	22	2
Cephalothin	16	0	24	1	23	2
Cephapirin	17	0	53	10	28	2

Resistant = MIC > 25 μg/ml, Sensitive = MIC ≤ 6.25 μg/ml

Table 2. Sensitivity of organisms resistant to BL-S217 to other cephalosporins

Organisms	Number resistant to BL-S217	Number sensitive to			
		Cephalexin	Cephaloridine	Cephalothin	Cephapirin
Klebsiella	11	4	0	0	0
P. mirabilis	14	0	1	1	0
E. coli	16	0	1	0	0

Resistant = MIC > 25 μg/ml, Sensitive = MIC ≤ 6.25 μg/ml

and indole-positive *Proteus* spp. at concentrations of 400 μg/ml. It also showed little activity against *S. marcescens* or *Enterobacter* spp. Over 90% of the isolates of these gram-negative bacilli were resistant to 400 μg/ml.

The effect of inoculum variation on the activity of BL-S217 was tested against 10 isolates each of *Klebsiella* spp. and *E. coli* (Fig. 3). The antibiotic was much less active against a 10^{-2} dilution of these isolates than against a 10^{-3} dilution. Further tenfold dilutions of 10^{-4} and 10^{-5} had less effect, especially for the isolates of *Klebsiella* spp.

The activity of BL-S217 was compared with cephalexin, cephaloridine, cephalothin and cephapirin. Cephaloridine and cephapirin were more active than BL-S217 against penicillin G sensitive *S. aureus* (Fig. 4). Cephalothin was more active than BL-S217 against the majority of these isolates. Cephalexin was substantially less active than the other cephalosporins against *S. aureus* whether sensitive or resistant to penicillin G (Fig. 5). BL-S217 was the most active cephalosporin against *D. pneumoniae* and cephalexin was the least active. BL-S217, cephaloridine, cephalothin and cephapirin were equally active against *S. pyogenes* (Fig. 6). Cephalexin was the least active antibiotic against all gram-positive cocci.

At a concentration of 12.5 μg/ml, cephalexin inhibited 90% of isolates of *Klebsiella* spp., BL-S217 inhibited 83%, cephaloridine inhibited 72%, cephalothin inhibited 69% and cephapirin inhibited 63% (Fig. 7). BL-S217, cephalexin, and cephaloridine were equally active against *E. coli*, inhibiting 77%, 76% and 71% of isolates respectively, at a concentration of 12.5 μg/ml (Fig. 8). At this concentration, cephalothin inhibited 59% of isolates of *E. coli* and cephapirin inhibited only 12%. Cephaloridine was the most active cephalosporin against *P. mirabilis*, inhibiting nearly 80% of isolates at a concentration of 12.5 μg/ml or less (Fig. 9). All of the other cephalosporins had similar activity although cephapirin was somewhat less active than the other antibiotics.

Table 1 shows the sensitivity of gram-negative bacilli which were resistant to the other cephalosporins to BL-S217. Of the 32 isolates of *E. coli*, *Klebsiella* sp. and *P. mirabilis* resistant to cephalexin, only one isolate of *P. mirabilis* was sensitive to BL-S217. Only 5 of 45 isolates resistant to cephaloridine and 3 of 63 isolates resistant to cephalothin were sensitive to BL-S217. Of the 41 isolates of *Klebsiella* sp., *P. mirabilis* and *E. coli* resistant to BL-S217, only 4 were sensitive to cephalexin, 2 were sensitive to cephaloridine and one was sensitive to cephalothin (Table 2).

Discussion

BL-S217 is a new cephalosporin antibiotic which is active *in vitro* against gram-positive cocci, including penicillin G resistant *S. aureus* and some gram-negative bacilli. In this study

the only gram-negative bacilli which were sensitive to BL-S217 were *E. coli*, *Klebsiella* sp. and *P. mirabilis*. BUCK *et al.* found that 37% of isolates of *Enterobacter* spp. were sensitive to BL-S217, whereas in this study only 4% were sensitive[3]. In subsequent studies, PRICE has found most isolates of *Enterobacter* spp. to be resistant to BL-S217[5]. Although BL-S217 was quite active against most isolates of *E. coli*, *Klebsiella* sp. and *P. mirabilis*, other cephalosporins were as active or more active. Consequently, from these *in vitro* studies it does not appear that BL-S217 offers any major advantage over other cephalosporin antibiotics.

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