ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1987, p. 653–656
0066-4804/87/040653-04$02.00/0

Vol. 31, No. 4

In Vitro Activity of BMY-28100, a New Oral Cephalosporin

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The activity of BMY-28100, a new orally administered cephalosporin, was compared with those of cephalexin and cefaclor. BMY-28100 was the most active drug against *Staphylococcus aureus* (MIC for 90% of strains tested [MIC_{90}], 1.0 μg/ml), streptococci (MIC_{90}s, ≤0.125 μg/ml), and *Klebsiella pneumoniae* (MIC_{90}, 2 μg/ml). The drug was active against *Haemophilus influenzae* and gonococci but not against other organisms generally resistant to cephem antibiotics.

BMY-28100 (Fig. 1) is a new 7-phenylglycyl cephalosporin which is being developed for oral administration. Preliminary data suggest that the new drug may be more resistant than cefaclor to hydrolysis by some beta-lactamases (M. Hiraoka, M. Inoue, S. Masuyoshi, K. Tomatsu, and S. Mitsuhashi, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 660, 1986). The present study examined the in vitro activity of BMY-28100 in comparison with those of cefaclor and cephalexin against more than 500 gram-positive and gram-negative bacterial isolates.

(This work was previously presented in part [G. M. Eliopoulos, E. Reiszner, C. Wennersten, and R. C. Moellering, Jr., Program Abstr. 26th ICAAC, abstr. no. 648, 1986].)

The gram-negative bacteria used in this study were, with few exceptions, recent clinical isolates collected at New England Deaconess Hospital, Boston, Mass. Most of the gram-positive organisms had been previously collected at Massachusetts General Hospital, Boston, except for penicillin-resistant strains of pneumococci and viridans group streptococci which had been obtained from South Africa (1, 3). Strains were kept frozen at −70°C until use. We performed susceptibility studies by an agar dilution technique (5) with Mueller-Hinton II agar (BBL Microbiology Systems, Cockeysville, Md.) which we supplemented with 5% defibrinated sheep blood when testing nonenterococcal streptococci and diphtheroids. We increased the agar concentration to 4% when testing *Proteus mirabilis* to prevent swarming. *Neisseria*, *Haemophilus*, and *Branhamella* strains were tested on GC agar base (BBL) supplemented with 1% hemoglobin and 1% IsoVitaleX enrichment (BBL) (5). We prepared inocula from overnight plate cultures by suspending several colonies in corresponding broth media to a cell density of ca. 10^7 CFU/ml and applied them with a multiprong device to yield final inocula of ca. 10^4 CFU. Plates were incubated in room air (except for gonococci and *Branhamella* spp., which were incubated in 5% CO_2) at 35°C for 18 to 20 h. Anaerobic bacteria were studied on Wilkens-Chalgren agar (Oxoid USA, Columbia, Md.); plates were read after 48 h of incubation in an anaerobic atmosphere generated by an anaerobic-system gas-generating kit (Oxoid).

Time-kill curve studies were carried out in 250-ml flasks (2). Volumes (20 ml) of Mueller-Hinton broth (BBL; for staphylococci) or brain heart infusion (Difco Laboratories, Detroit, Mich.; for *Haemophilus influenzae*) with 5% Fildes enrichment (Difco) were inoculated with suspensions prepared from overnight cultures to yield inocula of ca. 5×10^5 to 1×10^6 CFU/ml. Antibiotics were added at time zero at fixed multiples of the MIC. Samples of 0.5 ml were removed at 0, 4, and 24 h of incubation at 35°C in room air and serially diluted in normal saline. Colony counts were determined in duplicate, and the results were averaged.

Results of agar dilution susceptibility studies are shown in Tables 1 and 2. BMY-28100 was more active than either of the other agents against methicillin-susceptible strains of *Staphylococcus aureus* and *S. epidermidis* and all streptococci. Methicillin-resistant *S. aureus* was uniformly resistant to the new compound. Although BMY-28100 was the most active drug against enterococci, MICs for 90% of the strains tested (MIC_{90}s) against these organisms were ≥16 μg/ml, concentrations unlikely to be clinically achievable except perhaps in urine. Many strains of JK diphtheroids were highly resistant to these cephalosporins (MIC_{90}s, >128 μg/ml).

Activities of the three antimicrobial agents against *Escherichia coli* were comparable. BMY-28100 and cefaclor were superior to cephalexin against *Klebsiella pneumoniae* and *Proteus mirabilis*. *Citrobacter freundii*, *Enterobacter* spp., *Serratia marcescens*, and *Pseudomonas aeruginosa* were resistant to all of these agents (MIC_{90}, >128 μg/ml). BMY-28100 demonstrated poor activity against 10 strains each of *Pseudomonas maltophilia* and *Pseudomonas cepacia* (MIC_{90}, >128 μg/ml) and 30 strains of *Bacteroides fragilis* (all MICs, >32 μg/ml).

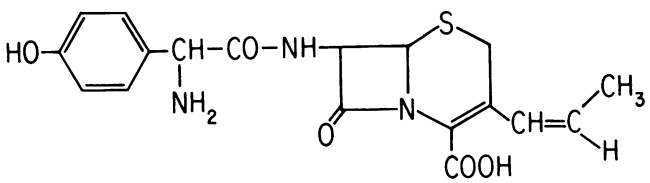

FIG. 1. Chemical structure of BMY-28100.

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TABLE 1. Comparative in vitro activity of BMY-28100

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (20)	BM-28100	0.125–2	0.5	1.0
	Cephalexin	4–16	4	16
	Cefaclor	2–16	4	8
Staphylococcus aureus, methicillin resistant (20)	BMY-28100	≥128	>128	>128
	Cephalexin	≥128	>128	>128
	Cefaclor	≥128	>128	>128
Staphylococcus epidermidis, methicillin susceptible (10)	BMY-28100	≤0.06–16	1.0	8
	Cephalexin	4–64	16	32
	Cefaclor	1.0–32	8	16
Staphylococcus epidermidis, methicillin resistant (20)	BMY-28100	≤0.06–16	8	16
	Cephalexin	2–64	64	64
	Cefaclor	1.0–64	64	64
Streptococcus faecalis (30)	BMY-28100	4–16	8	16
	Cephalexin	32–128	128	128
	Cefaclor	8–64	64	64
Streptococcus faecium (10)	BMY-28100	16–64	16	64
	Cephalexin	≥128	>128	>128
	Cefaclor	32–128	64	128
Streptococcus avium (10)	BMY-28100	2–32	2	32
	Cephalexin	1.0–128	32	128
	Cefaclor	4–64	16	64
Streptococcus pneumoniae, penicillin susceptible (8)	BMY-28100	≤0.06		≤0.06
	Cephalexin	1.0–2		2
	Cefaclor	0.25–0.5		0.5
Streptococcus pneumoniae, penicillin resistant (10)	BMY-28100	0.5–4	4	4
	Cephalexin	4–64	16	64
	Cefaclor	2–64	8	64
Group A streptococci (10)	BMY-28100	≤0.06	≤0.06	≤0.06
	Cephalexin	0.5	0.5	0.5
	Cefaclor	0.25	0.25	0.25
Group B streptococci (10)	BMY-28100	≤0.06–0.125	0.125	0.125
	Cephalexin	1.0–4	4	4
	Cefaclor	0.5–2	2	2
Group C streptococci (5)	BMY-28100	≤0.06		
	Cephalexin	0.5–1.0		
	Cefaclor	0.25		
Group G streptococci (5)	BMY-28100	≤0.06–0.125		
	Cephalexin	1.0		
	Cefaclor	0.25–0.5		
Viridans group streptococci, penicilllin susceptible (20)	BMY-28100	≤0.06–0.25	0.125	0.125
	Cephalexin	0.5–32	4	8
	Cefaclor	0.5–16	2	4
Viridans group streptococci, penicillin resistant (10)	BMY-28100	2–64	8	32
	Cephalexin	64–>128	>128	>128
	Cefaclor	≥128	>128	>128
Listeria monocytogenes (10)	BMY-28100	4–8	4	4
	Cephalexin	32–128	64	64
	Cefaclor	16–32	16	16
JK corynebacteria (10)	BMY-28100	4–>128	8	>128
	Cephalexin	32–>128	64	>128
	Cefaclor	32–>128	32	>128

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli (30)	BMY-28100	0.25–16	2	8
	Cephalexin	4–16	8	16
	Cefaclor	0.5–8	2	4
Klebsiella pneumoniae (40)	BMY-28100	0.25–8	1.0	2
	Cephalexin	4–32	8	16
	Cefaclor	0.5–8	2	8
Citrobacter freundii (25)	BMY-28100	4–>128	16	>128
	Cephalexin	16–>128	>128	>128
	Cefaclor	16–>128	128	>128
Enterobacter spp. (55)	BMY-28100	4–>128	128	>128
	Cephalexin	16–>128	>128	>128
	Cefaclor	16–>128	>128	>128
Serratia marcescens (20)	BMY-28100	≥128	>128	>128
	Cephalexin	>128	>128	>128
	Cefaclor	>128	>128	>128
Proteus mirabilis (30)	BMY-28100	1.0–4	1.0	1.0
	Cephalexin	16–32	16	16
	Cefaclor	1.0–4	1.0	2
Morganella morganii (10)	BMY-28100	128–>128	>128	>128
	Cephalexin	16–>128	>128	>128
	Cefaclor	4–>128	>128	>128
Proteus vulgaris (10)	BMY-28100	>128	>128	>128
	Cephalexin	>128	>128	>128
	Cefaclor	>128	>128	>128
Pseudomonas aeruginosa (30)	BMY-28100	>128	>128	>128
	Cephalexin	>128	>128	>128
	Cefaclor	>128	>128	>128

BMY-28100 inhibited both β-lactamase-producing and β-lactamase-negative strains of *H. influenzae* at comparable concentrations (2 to 16 μg/ml) (Table 2). These results are similar to those reported by other investigators (L. P. Smith and I. M. Gladstone, 26th ICAAC, abstr. no. 656, 1986). Because activities of antibiotics against fastidious microorganisms may be influenced by the methods used, the activities of BMY-28100 and cefaclor against *H. influenzae* were examined by an alternative technique, the suggested National Committee for Clinical Laboratory Standards method for susceptibility testing of fastidious organisms (microdilution test in cation-supplemented Mueller-Hinton broth containing 2% lysed horse blood and 1% IsoVitaleX; inoculum, 5×10^5 CFU/ml) (4). Against 17 β-lactamase-negative strains, the respective MIC_{90}s of BMY-28100 and cefaclor were 4 and 8 μg/ml (ranges, 1.0 to 8 and 2 to 16 μg/ml, respectively). Against seven β-lactamase-producing isolates, MICs of BMY-28100 ranged from 1.0 to 16 μg/ml, whereas MICs of cefaclor ranged from 4 to 32 μg/ml. β-Lactamse-producing *N. gonorrhoeae* were less susceptible to BMY-28100 than were β-lactamase-negative strains (Table 2). Three of four strains of *B. catarrhalis* tested produced β-lactamase detected with nitrocefin. These strains had higher MICs (4 μg/ml) than did the β-lactamase-negative strain (1.0 μg/ml).

TABLE 2. Activity of BMY-28100 against *H. influenzae*, *N. gonorrhoeae*, and *B. catarrhalis*

Organism (no. of isolates)[a]	MIC (μg/ml)		
	Range	50%	90%
H. influenzae (BLA−) (10)	2–16	4	16
H. influenzae (BLA+) (10)	2–16	4	8
N. gonorrhoeae (BLA−) (9)	0.25–8		2
N. gonorrhoeae (BLA+) (9)	4–16		16
B. catarrhalis (4)	1.0–4		

[a] BLA, β-Lactamase negative (−) or positive (+).

The bactericidal activity of BMY-28100 is illustrated in Fig. 2. A higher multiple of the MIC was required to achieve a bactericidal effect against a β-lactamase-producing *H. influenzae* comparable to that observed against a β-lactamase-negative strain (panels A and B). Results shown in Fig. 2 indicate that BMY-28100 is unlikely to exert a reliable bactericidal effect against β-lactamase-producing isolates of these species at readily attainable concentrations in serum. The magnitudes of killing at 24 h of incubation with BMY-28100 or cefaclor, each at four times the MIC, were compared for the four strains represented in Fig. 2. Rounded to the nearest 0.5 $\log_{10}$ CFU/ml, the killing results (decrease in CFU/ml) with BMY-28100 and cefaclor, respectively, were as follows: *H. influenzae* T1, 4.5 and 4.5; *H. influenzae*

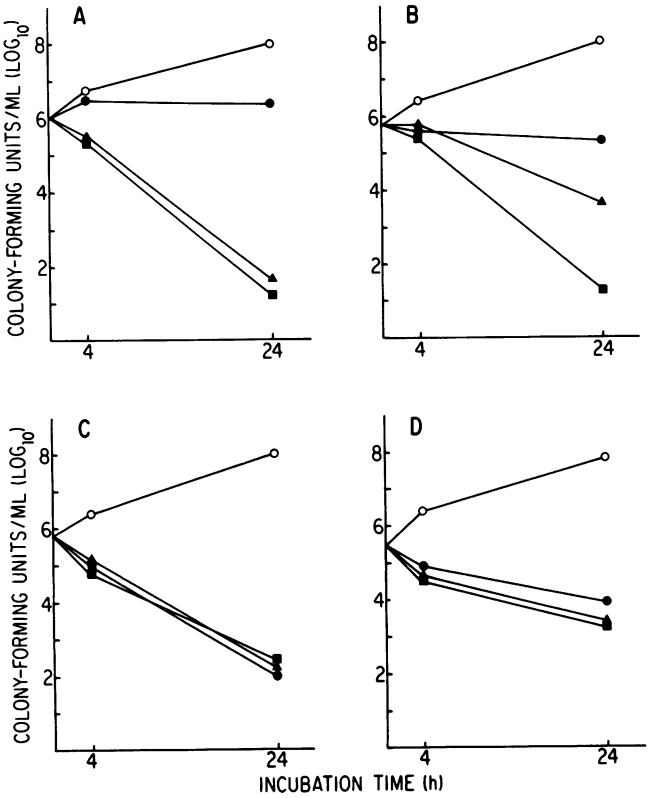

FIG. 2. Bactericidal activity of BMY-28100 at twice (●), four times (▲), and eight times (■) the MIC. Open circles (○) represent control flasks without antibiotic. Panels: A, *H. influenzae* T1 (β-lactamase negative) with BMY-28100 at 4, 8, and 16 μg/ml; B, *H. influenzae* T21 (β-lactamase producing) with BMY-28100 at 8, 16, and 32 μg/ml; C, *S. aureus* 1 with BMY-28100 at 1, 2, and 4 μg/ml; D, *S. aureus* 2 with BMY-28100 at 2, 4, and 8 μg/ml. Both strains of *S. aureus* produce β-lactamase.

T21, 2.0 and 4.0; *S. aureus* 1, 3.5 and 3.5; and *S. aureus* 2, 2 and 1.0.

BMY-28100 was more active than either cefaclor or cephalexin against *S. aureus*, streptococci, and *K. pneumoniae*. It is not clear whether these differences are of sufficient magnitude to be clinically important. The new drug was also active against *H. influenzae* and *N. gonorrhoeae*, but some strains may be relatively resistant as determined by the methods used in this study.

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