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Comparative Antibacterial Activity of a New Oral Cephalosporin, BMY-28100

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BMY-28100 is a new oral cephalosporin which had in vitro activity superior to that of cephalexin and cefaclor against staphylococci, beta-hemolytic streptococcal species, and *Streptococcus pneumoniae*. It inhibited beta-lactamase-producing *Haemophilus influenzae*, *Neisseria gonorrhoeae*, 50% of *Streptococcus faecalis* isolates, *Listeria monocytogenes*, and 50 to 75% of *Escherichia coli* and *Klebsiella* species at ≤8 μg/ml, but high producers of beta-lactamase were resistant. *Enterobacter*, *Citrobacter*, *Morganella*, *Providencia*, and *Pseudomonas* species and *Bacteroides fragilis* were resistant. BMY-28100 was more stable than cefaclor against hydrolysis by beta-lactamases.

There is continued interest in the development of new oral antimicrobial agents that can be used to treat infections caused by gram-positive and gram-negative bacteria (3). The available orally administered cephalosporin compounds, such as cephalexin, cephradine, and cefaclor, have relatively short half-lives and generally are administered four times a day. BMY-28100 is a new semisynthetic cephalosporin which contains a 4-hydroxyphenyl group on the beta acyl side chain and a 1-propenyl group at position 3 of the bicyclic nucleus. We wished to compare the antibacterial activity of BMY-28100 with the activity of other oral cephalosporins and penicillins.

Most of the isolates used in this study were recent clinical isolates from patients seen at the Columbia-Presbyterian Medical Center, New York, N.Y.

Standard test powders were provided as follows: BMY-28100, Bristol-Myers Laboratories, Syracuse, N.Y.; cephalexin and cefaclor, Eli Lilly & Co., Indianapolis, Ind.; amoxicillin and amoxicillin-clavulanate, Beecham Laboratories, Bristol, Tenn. All compounds were prepared on the day of use.

Susceptibility testing was performed by a standard agar dilution technique with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% defibrinated sheep blood for streptococci. Brucella agar supplemented with 5% laked sheep blood, hemin, and vitamin K was used for anaerobic species. Chocolatized agar supplemented with IsoVitaleX (BBL) was used for *Haemophilus influenzae* and *Neisseria gonorrhoeae*. A final inoculum of 10^5 CFU was applied to plates by using a multiprong inoculator. Aerobic plates were incubated for 18 h at 35°C. Anaerobic plates were incubated in GasPak (BBL) containers for 48 h at 35°C. For testing methicillin-resistant staphylococci, 3% NaCl was added to the medium. All drugs were tested simultaneously.

Broth dilution studies were performed with a 1-ml volume of medium containing a final inoculum of 5×10^5 CFU/ml. Todd-Hewitt broth was used for streptococci. Mueller-Hinton broth was used for *Staphylococcus aureus*, and Schaedler broth was used for *H. influenzae* and *Branhamella catarrhalis*. After incubation for 18 to 20 h at 35°C, samples of 0.01 ml were removed to antibiotic-free plates and incubated at 35°C for 24 h. The MBC was defined as the concentration giving a 99.9% reduction in the initial inoculum.

Rate of kill experiments were performed with exponential-phase organisms at an inoculum of 5×10^5 to 10^6 CFU in Mueller-Hinton broth. Samples were removed at hourly intervals and plated on antibiotic-free plates to determine the CFU.

The presence of beta-lactamases in isolates was determined by the nitrocefin spot assay (2). The beta-lactamases used were previously described (2). Stability against beta-lactamases was determined by a spectrophotometer assay by measuring the change in the A_{265} for cephalexin, the A_{267} for cefaclor, and the A_{265} for BMY-28100 (2). Cephaloridine at 260 nm at a concentration of 10^{-4} M was used as a standard of 100% hydrolysis. Activity was calculated as micromoles per milliliter of enzyme. The rates of hydrolysis with different enzymes cannot be directly compared, because the specific activities of the preparations differed.

The overall activity of BMY-28100 compared with those of other oral agents is shown in Table 1. BMY-28100 was slightly more active than cephalexin and cefaclor against *S. aureus* but less active than amoxicillin-clavulanate. It did not inhibit methicillin-resistant *S. aureus*. BMY-28100 was appreciably more active against methicillin-susceptible *Staphylococcus epidermidis* than the other two cephalosporins. For *Streptococcus pyogenes*, BMY-28100 was significantly more active than the other cephalosporins, with 90% inhibited at 0.12 μg/ml, as compared with cefaclor at 1 μg/ml and cephalexin at 2 μg/ml. It was comparable in activity to amoxicillin. Although BMY-28100 was more active than the other cephalosporins for many of the isolates of the other hemolytic streptococci, groups B, C, F, and G, and *Streptococcus bovis*, for each group of organisms there were some isolates for which MICs were 4 to 8 μg/ml. However, 0.25 μg of BMY-28100 per ml inhibited 50% of beta-hemolytic streptococci. Interestingly, BMY-28100 inhibited 50% of the *Streptococcus faecalis* isolates at 8 μg/ml, whereas the MICs of cephalexin and cefaclor were >32 μg/ml. BMY-28100 was two- to fourfold more active than the other cephalosporins

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TABLE 1. In vitro activity of BMY-28100 compared with that of other oral agents

Organism (no. of isolates)	Agent	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus (methicillin susceptible) (30)	BMY-28100	0.25–8	1	4
	Cephalexin	0.5–16	2	8
	Cefaclor	0.5–16	2	8
	Amoxicillin	0.5–16	1	8
	Amox-clavulanate[b]	0.25–2	0.25	1
Staphylococcus aureus (methicillin resistant) (15)	BMY-28100	1–>32	4	32
	Cephalexin	2–>32	8	>32
	Cefaclor	2–>32	>32	>32
	Amoxicillin	1–>32	16	>32
	Amox-clavulanate	0.5–>16	1	16
Staphylococcus epidermidis (methicillin susceptible) (25)	BMY-28100	0.5–8	0.25	0.5
	Cephalexin	0.5–>32	1	8
	Cefaclor	0.5–4	1	4
	Amoxicillin	0.5–8	0.5	4
	Amox-clavulanate	0.12–4	0.25	1
Staphylococcus epidermidis (methicillin resistant) (15)	BMY-28100	1–>32	4	16
	Cephalexin	8–>32	>32	>32
	Cefaclor	8–>32	>32	>32
	Amoxicillin	4–>32	>32	>32
	Amox-clavulanate	0.5–>16	2	>16
Staphylococcus saprophyticus (10)	BMY-28100	0.25–1	0.5	1
	Cephalexin	1–8	2	4
	Cefaclor	1–4	1	2
Streptococcus pyogenes (25)	BMY-28100	0.03–0.25	0.03	0.12
	Cephalexin	0.06–2	0.5	2
	Cefaclor	0.06–1	0.5	1
	Amoxicillin	0.03–0.25	0.03	0.12
	Amox-clavulanate	0.03–0.25	0.03	0.12
Streptococcus agalactiae (30)	BMY-28100	0.06–4	0.12	1
	Cephalexin	0.5–4	1	4
	Cefaclor	0.5–4	0.5	4
	Amoxicillin	0.03–0.5	0.12	0.5
	Amox-clavulanate	0.12–0.5	0.12	0.5
Streptococcus bovis (25)	BMY-28100	0.12–8	0.12	8
	Cephalexin	0.5–8	1	8
	Cefaclor	0.12–8	0.25	8
	Amoxicillin	0.12–0.5	0.12	0.25
	Amox-clavulanate	0.12–0.25	0.12	0.12
Streptococcus group C (15)	BMY-28100	0.03–2	0.06	1
	Cephalexin	0.12–4	0.25	4
	Cefaclor	0.12–1	0.12	1
	Amoxicillin	0.03–0.5	0.03	0.25
	Amox-clavulanate	0.03–0.25	0.03	0.25
Streptococcus groups F and G (24)	BMY-28100	0.03–8	0.25	2
	Cephalexin	0.12–8	1	8
	Cefaclor	0.12–8	0.5	8
	Amoxicillin	0.03–0.25	0.03	0.25
	Amox-clavulanate	0.03–0.5	0.06	0.25
Streptococcus faecalis (30)	BMY-28100	8–16	8	16
	Cephalexin	≥32	32	>32
	Cefaclor	8–>32	8	>32
	Amoxicillin	0.12–0.5	0.12	0.25
	Amox-clavulanate	0.12–0.5	0.25	0.5
Viridans group streptococci (19)	BMY-28100	0.25–32	4	16
	Cephalexin	0.5–32	16	>32
	Cefaclor	0.5–>32	8	>32
	Amoxicillin	0.12–4	0.12	1
	Amox-clavulanate	0.12–2	0.12	0.5
Streptococcus pneumoniae (30)	BMY-28100	0.12–0.5	0.12	0.5
	Cephalexin	0.5–8	1	2
	Cefaclor	0.25–2	0.25	1
	Amoxicillin	0.03–0.12	0.03	0.12
	Amox-clavulanate	0.03–0.12	0.03	0.12
Listeria monocytogenes (20)	BMY-28100	2–32	4	16
	Cephalexin	16–>32	>32	>32
	Cefaclor	8–>32	8	16
	Amoxicillin	0.12–0.5	0.25	0.5

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TABLE 1—*Continued*

Organism (no. of isolates)	Agent	MIC (μg/ml)[a]		
		Range	50%	90%
Haemophilus influenzae (20)	BMY-28100	0.5–8	1	2
	Cephalexin	1–16	4	16
	Cefaclor	0.25–16	2	4
	Amoxicillin	0.12–>16	0.5	>16
	Amox-clavulanate	0.12–1	0.25	1
Branhamella catarrhalis (16)	BMY-28100	0.5–8	1	2
	Cephalexin	0.5–16	2	4
	Cefaclor	0.5–16	1	2
	Amoxicillin	0.25–16	2	16
	Amox-clavulanate	0.12–1	0.25	0.5
Neisseria gonorrhoeae (14)	BMY-28100	0.5–16	1	8
	Cephalexin	0.5–16	4	16
	Cefaclor	0.5–16	2	16
	Amoxicillin	0.12–16	8	>16
	Amox-clavulanate	0.12–2	0.25	1
Escherichia coli (40)	BMY-28100	1–>128	2	8
	Cephalexin	4–>128	8	16
	Cefaclor	2–>128	8	>128
	Amoxicillin	4–>128	8	>128
	Amox-clavulanate	1–8	4	8
Klebsiella pneumoniae (30)	BMY-28100	0.25–>64	4	64
	Cephalexin	4–>64	8	>64
	Cefaclor	2–>64	4	>64
	Amoxicillin	>64	>64	>64
	Amox-clavulanate	2–16	2	16
Klebsiella oxytoca (20)	BMY-28100	1–>64	8	>64
	Cephalexin	1–>64	8	>64
	Cefaclor	1–>64	8	>64
	Amoxicillin	>64	>64	>64
	Amox-clavulanate	2–16	2	16
Proteus mirabilis (30)	BMY-28100	0.5–>128	1	1
	Cephalexin	2–>128	4	16
	Cefaclor	1–>128	1	2
	Amoxicillin	0.5–>128	1	2
	Amox-clavulanate	0.5–8	1	2
Citrobacter diversus (20)	BMY-28100	0.5–8	1	2
	Cephalexin	4–32	4	8
	Cefaclor	4–32	2	8
	Amoxicillin	>128	>128	>128
	Amox-clavulanate	1–32	8	32
Salmonella spp. (25)	BMY-28100	1–>128	4	>128
	Cephalexin	4–32	8	32
	Cefaclor	2–128	8	>128
	Amoxicillin	1–>128	>128	>128
	Amox-clavulanate	1–8	2	8
Shigella spp. (25)	BMY-28100	2–64	8	32
	Cephalexin	2–64	8	32
	Cefaclor	2–64	8	32
	Amoxicillin	2–>128	16	>128
	Amox-clavulanate	1–8	2	8
Clostridium spp. (15)	BMY-28100	0.06–4	0.12	1
	Cephalexin	2–>64	16	16
	Cefaclor	2–>64	8	16
	Amoxicillin	0.12–2	0.25	2
	Amox-clavulanate	0.12–0.5	0.12	0.5
Enterobacter aerogenes (20)	BMY-28100	32–>128	>128	>128
Enterobacter agglomerans (10)	BMY-28100	1–>128	16	>128
Enterobacter cloacae (20)	BMY-28100	32–>128	128	>128
Morganella morganii (10)	BMY-28100	4–>128	128	>128
Proteus vulgaris (10)	BMY-28100	4–>128	>128	>128
Providencia stuartii (10)	BMY-28100	1–>128	32	>128
Pseudomonas aeruginosa (10)	BMY-28100	>128	>128	>128
Acinetobacter anitratus (10)	BMY-28100	32–>128	128	>128
Serratia marcescens (10)	BMY-28100	>128	>128	>128
Bacteroides fragilis (15)	BMY-28100	16–>128	64	>128

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.
[b] Amox, Amoxicillin. Amoxicillin-clavulanate was present at two parts amoxicillin, one part clavulanate. Numbers represent amoxicillin concentrations.

TABLE 2. Stability of BMY-28100 against hydrolysis by beta-lactamases

Beta-lactamase	Bacterial source	Richmond-Sykes classification	Amt hydrolyzed (μmol/ml)			
			Cephaloridine	BMY-28100	Cefaclor	Cephalexin
TEM-1	*Escherichia coli*	IIIa	16	3.2	8.5	<0.1
SHV-1	*Klebsiella pneumoniae*	IIIa	54.4	11	57.1	3.4
K-1	*Klebsiella oxytoca*	IV	1,076	367	939	167
P99	*Enterobacter cloacae*	Ia	1,404	204	1,052	208
	Proteus vulgaris	Ic	520	102	973	235
Sabath-Abraham	*Pseudomonas aeruginosa*	Id	80	16	89.1	30

against *Streptococcus pneumoniae* but less active than amoxicillin. BMY-28100 was more active than cefaclor against *H. influenzae* and of equal activity against *B. catarrhalis*, inhibiting amoxicillin-resistant isolates, but it was not more active than the combination of amoxicillin and clavulanate.

The activity of BMY-28100 against *Escherichia coli* and *Klebsiella pneumoniae* was related to the presence of beta-lactamases. Those isolates with high production of beta-lactamase, as determined by immediate reaction of nitrocefin, had higher MICs. However, BMY-28100 at ≤8 μg/ml did inhibit most of the *E. coli* isolates that were resistant to amoxicillin. Eighty percent of *E. coli* and *Klebsiella* spp. isolates were inhibited by ≤8 μg/ml. Amoxicillin-clavulanate had activity comparable to that of BMY-28100 against both *E. coli* and the *Klebsiella* species. Both *Proteus mirabilis* and *Citrobacter diversus* were inhibited by BMY-28100, which was more active than cephalexin or cefaclor and comparable in activity to amoxicillin-clavulanate. Although BMY-28100 inhibited 50% of the *Salmonella* and *Shigella* spp., 20% had MICS ≥32 μg/ml. These isolates were high producers of beta-lactamase. BMY-28100 had an MIC of >16 μg/ml for *Enterobacter* species, *Morganella morganii*, *Proteus vulgaris*, *Providencia stuartii*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Acinetobacter anitratus*, and *Bacteroides fragilis*. *Clostridium* spp. were inhibited, and BMY-28100 was more active than cefaclor or cephalexin against these anaerobes.

Effect of growth conditions. BMY-28100 had similar activity against staphylococci, *S. faecalis*, *E. coli*, and *K. pneumoniae* at pH 5.6, 6.5, and 7.5. Likewise, there was no difference in activity with Mueller-Hinton, brain heart infusion, and tryptic soy digest agar media. An inoculum size effect was noted when the inoculum was increased from 10^5 to 10^7 CFU for *S. aureus*, *S. epidermidis*, and beta-lactamase-producing *E. coli*, *K. pneumoniae*, and *Klebsiella oxytoca* (five isolates each). The MICs at 10^7 CFU were eightfold greater than at 10^5 CFU.

Rate of kill activity. BMY-28100 at twice the MIC for *S. aureus*, 0.5 μg/ml, produced a 1-$\log_{10}$ decrease in CFU in 4 h and a 2.3-$\log_{10}$ decrease in 8 h, with regrowth at 24 h. At eight times the MIC, the decrease in CFU was similar, but there was a 3.4-$\log_{10}$ decrease in 24 h without regrowth. With *E. coli* (MIC, 4 μg/ml), at the MIC there was a 2-$\log_{10}$ decrease in CFU at 4 h and a 3-$\log_{10}$ decrease in CFU at 8 h without regrowth at 24 h.

Beta-lactamase stability. The stability of BMY-28100 against beta-lactamases was compared with those of cefaclor and cephalexin (Table 2). BMY-28100 was hydrolyzed to a degree by all of the enzymes. It was more stable than cefaclor for the most commonly encountered plasmid enzyme, TEM-1, with a relative rate of 20 as compared with 53, with cephaloridine set as 100%, but it was less stable than cephalexin. With the chromosomal beta-lactamase of the Richmond-Sykes Ia type of *Enterobacter cloacae*, it was as stable as cephalexin, and it was more stable than cephalexin with the Sabath-Abraham *Pseudomonas* enzyme of the Richmond-Sykes Id type and the Ic enzymes of *P. vulgaris*.

BMY-28100 was more active than cefaclor or cephalexin against methicillin-susceptible staphylococci and against beta-hemolytic streptococci. It had slightly increased in vitro activity against *S. pneumoniae* and some *H. influenzae* isolates as compared with the other two cephalosporins, but it was not as active as the amoxicillin-clavulanate combination for many of the gram-negative bacteria tested. BMY-28100 was also more beta-lactamase stable than cefaclor and similar in stability to cephalexin. These studies showed higher MICs than have been published (1) for cephalexin and cefaclor, which is probably related to the inclusion of many hospital-acquired isolates.

Although BMY-28100 inhibited many *E. coli*, *Klebsiella* species, and *P. mirabilis* isolates at ≤8 μg/ml, the breakpoint for the other cephalosporins, some *E. coli* and *Klebsiella* isolates were resistant, and it did not inhibit *Enterobacter* spp., *Citrobacter freundii*, or *S. marcescens* and had no activity against *B. fragilis*. Also, BMY-28100 did not inhibit *K. pneumoniae* or *K. oxytoca* isolates that were resistant to cephalexin. Based on these data, BMY-28100 has excellent potential as an agent for organisms causing upper respiratory and skin-structure infections, provided its pharmacokinetic properties are unsatisfactory. In considering its role for enteric species, if levels in blood with BMY-28100 are significantly lower than those with other oral cephalosporins and a lower breakpoint is necessary, BMY-28100 would have to be considered useful primarily for non-beta-lactamase-producing members of the family *Enterobacteriaceae*. Further investigation of this oral agent will depend on its pharmacological and toxicological properties.

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