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Evaluation of the In Vitro Activity of BMY-28142, a New Broad-Spectrum Cephalosporin

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The in vitro activity of BMY-28142, a new cephalosporin, was tested by a broth microdilution system and compared with those of cefotaxime, ceftazidime, cefoperazone, moxalactam, and HR 810 against 747 bacterial isolates, one-third of which were resistant to one or more third-generation cephalosporins. BMY-28142 was the most active drug tested against 326 *Enterobacteriaceae* with an MIC for 90% of the organisms tested (MIC_{90}) of 1.0 μg/ml. Against these *Enterobacteriaceae* the relative activities were: BMY-28142 > HR 810 > moxalactam and ceftazidime > cefotaxime > cefoperazone. For cefotaxime- and cefoperazone-resistant strains, the MIC_{90} of BMY-28142 was 4.0 μg/ml (compared with 0.13 μg/ml for susceptible strains). BMY-28142, with an MIC_{90} of 8.0 μg/ml for *Pseudomonas aeruginosa*, was about half as active as ceftazidime. The relative activities against *P. aeruginosa* were: ceftazidime > BMY-28142 > HR 810 > cefoperazone > moxalactam and cefotaxime. The MIC_{90} of BMY-28142 against staphylococci was 2.0 μg/ml, which was fourfold less active than HR 810, slightly less active than cefotaxime and cefoperazone, and fourfold more active than ceftazidime and moxalactam. BMY-28142 was very active against β-lactamase-positive and -negative *Haemophilus influenzae* (MIC_{90}, 0.06 μg/ml), *Neisseria gonorrhoeae* (MIC_{90}, 0.015 μg/ml), and nonenterococcal streptococci. Its activity against *Streptococcus faecalis* was poor (MIC_{90}, 64 μg/ml). BMY-28142 was stable against the several β-lactamases tested but exhibited little β-lactamase inhibitory effect.

BMY-28142 is a new parenteral cephalosporin with the structure 7-[α-(2-aminothiozol-4-yl)-α-(7)-methoximino-acetamido] - 3 - (1 - methylpyrrolidinio) - methyl - 3 - cephem-4-carboxylate. In preliminary studies it has demonstrated excellent activity against the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, staphylococci, and streptococcal species other than enterococci (6; M. Bies, R. E. Buck, T. A. Pursiano, D. R. Chisholm, Y. H. Tsai, M. Misiek, K. E. Price, and F. Leitner, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 577, 1983).

In this study, we compared the in vitro activity of BMY-28142 in a broth microdilution system with those of four third-generation cephalosporins: cefotaxime, ceftazidime, cefoperazone, and moxalactam, as well as with HR 810, a structurally similar and potent new cephalosporin (4). The β-lactamase stability of BMY-28142 and β-lactamase inhibition by this drug are also reported.

MATERIALS AND METHODS

Antimicrobial agents. BMY-28142 and dicloxacillin were provided by Bristol Laboratories, Syracuse, N.Y. The comparison drugs cefotaxime and HR 810 were obtained from Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.; ceftazidime and nitrocefin were supplied by Glaxo, Inc., Research Triangle Park, N.C.; moxalactam and cephaloridine were from Eli Lilly & Co., Indianapolis, Ind.; and cefoperazone and sulbactam came from Pfizer Inc., New York, N.Y.

Bacteria. A total of 747 bacterial isolates representing 36 species were tested (Table 1). The majority were recent clinical isolates collected from the microbiology laboratories of the Cleveland Clinic Foundation, Cleveland, Ohio; the Kaiser-Permanente Health Care Program Regional Laboratory, Clackamas, Oreg.; Northwestern Memorial Hospital, Chicago, Ill.; St. Francis Hospital, Wichita, Kans.; and St. Vincent Hospital and Medical Center, Portland, Oreg. Nearly one-third of the isolates tested were specifically selected because of known resistance (MIC ≥ 16 μg/ml) to one or more third-generation cephalosporins.

Susceptibility testing. For most organisms, MICs were determined by broth microdilution methods as described by the National Committee for Clinical Laboratory Standards (7) and in previous studies (2–4). For testing nonenterococcal streptococci and *Neisseria gonorrhoeae*, an agar dilution method was used, which has been described previously in more detail (1). A sample of 106 nonfastidious organisms were also tested simultaneously by both agar dilution and broth microdilution methods to assess the comparability of the results of the two methods (7). The drug concentrations tested were serial twofold increments ranging from 0.008 to 256 μg/ml for BMY-28142 and HR 810 and from 0.06 to 32 μg/ml for cefotaxime, cefoperazone, moxalactam, and ceftazidime. The lowest concentration inhibiting the growth of an inoculum containing about 5×10^5 CFU/ml after 16 to 20 h of aerobic incubation at 35°C was considered the MIC in broth microdilution tests. For agar dilution, the inoculum contained about 10^4 CFU per spot.

β-Lactamase studies. The β-lactamase stability of BMY-28142 as well as its inhibiting effect on various β-lactamases were determined by previously described methods (3, 5). Briefly, a 100 μM solution of BMY-28142 (or other β-lactam substrate) in 0.05 M phosphate buffer (pH 7) was reacted with crude enzyme extracts prepared by the method of Neu (8) from organisms known to produce β-lactamase

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TABLE 1. Susceptibility of 747 bacterial isolates to BMY-28142 and three other cephalosporins

Organism	No. of isolates	BMY-28142		HR 810		Ceftazidime		Cefotaxime	
		MIC_{50}[a]	MIC_{90}[a]	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Citrobacter diversus	10	0.03	0.03	0.03	0.06	0.12	0.12	≤0.06	0.12
Citrobacter freundii	18	0.5	4.0	1.0	8.0	>32	>32	32	>32
Enterobacter aerogenes	43	0.06	0.5	0.06	1.0	1.0	>32	2.0	32
Enterobacter agglomerans	10	0.03	0.5	0.03	0.5	0.25	1.0	0.12	1.0
Enterobacter cloacae	47	0.25	4.0	0.25	16	16	>32	32	>32
Escherichia coli	34	0.03	0.06	0.03	0.06	0.12	0.25	≤0.06	≤0.06
Klebsiella pneumoniae	24	0.03	0.06	0.03	0.25	0.12	0.25	≤0.06	≤0.06
Proteus mirabilis	26	0.03	0.06	0.03	0.06	≤0.06	≤0.06	≤0.06	≤0.06
Proteus vulgaris	10	0.03	0.5	0.12	2.0	≤0.06	0.12	≤0.06	32
Providencia rettgeri	10	0.015	0.5	0.015	0.25	≤0.06	1.0	≤0.06	0.5
Providencia stuartii	19	0.03	0.06	0.12	0.5	0.25	1.0	≤0.06	0.5
Serratia marcescens	41	0.13	1.0	0.06	2.0	0.25	2.0	0.5	32
Other *Enterobacteriaceae*[b]	34	0.03	0.5	0.03	0.5	0.12	1.0	≤0.06	2.0
All *Enterobacteriaceae*	326	0.03	1.0	0.03	2.0	0.12	>32	0.12	32
Acinetobacter calcoaceticus	17	0.5	4.0	1.0	4.0	2.0	4.0	8.0	32
Pseudomonas aeruginosa	69	2.0	8.0	4.0	16	2.0	4.0	32	>32
Pseudomonas flourescens	16	4.0	32	8.0	64	4.0	>32	>32	>32
Other *Pseudomonas* spp.[c]	25	2.0	16	2.0	64	1.0	4.0	4.0	32
Haemophilus influenzae									
β-Lactamase negative	19	≤0.008	0.06	≤0.008	0.03	≤0.06	≤0.06	≤0.06	≤0.06
β-Lactamase positive	20	0.03	0.06	0.015	0.25	≤0.06	≤0.06	≤0.06	≤0.06
Neisseria gonorrhoeae									
β-Lactamase negative	27	≤0.008	≤0.008	NT[d]	NT	NT	NT	≤0.06	≤0.06
β-Lactamase positive	25	≤0.008	0.015	NT	NT	NT	NT	≤0.06	≤0.06
Neisseria meningitidis	20	≤0.008	≤0.008	≤0.008	≤0.008	≤0.06	≤0.06	≤0.06	≤0.06
Staphylococcus aureus									
β-Lactamase negative	28	2.0	2.0	0.5	0.5	4.0	8.0	1.0	2.0
β-Lactamase positive	30	2.0	2.0	0.5	1.0	8.0	8.0	2.0	2.0
Methicillin resistant	12	64	128	16	32	>32	>32	>32	>32
Coagulase-negative staphylococci									
β-Lactamase negative	11	0.5	1.0	0.5	1.0	4.0	32	1.0	1.0
β-Lactamase positive	17	2.0	128	1.0	16	8.0	>32	4.0	>32
Streptococcus agalactiae	20	0.06	0.06	0.03	0.06	0.25	0.5	≤0.06	≤0.06
Streptococcus faecalis	25	32	64	8.0	16	>32	>32	>32	>32
Streptococcus pneumoniae	20	0.06	0.5	0.06	0.25	0.5	8.0	≤0.06	0.25
Streptococcus pyogenes	20	0.015	0.015	≤0.008	≤0.008	0.12	0.12	≤0.06	≤0.06

[a] MIC_{50} and MIC_{90}: lowest concentration (μg/ml) inhibiting 50 and 90% of isolates, respectively.
[b] Includes *Klebsiella oxytoca* (6), *Morganella morganii* (8), *Salmonella enteritidis* (9), *Serratia liquefaciens* (2), and *Shigella* spp. (9).
[c] Includes *P. acidovorans* (3), *P. cepacia* (4), *P. maltophilia* (4), *P. putida* (5), and *P. stutzeri* (9).
[d] Not tested.

types I to V (9). One commercially prepared (BBL Microbiology Systems, Cockeysville, Md.) β-lactamase derived from *Bacillus cereus* was also studied. The reaction mixtures were monitored for 20 min on a scanning UV spectrophotometer (model 552; The Perkin-Elmer Corp., Norwalk, Conn.) at wavelengths of 258 to 482 nm. Nitrocefin and cefaloridin served as the reference labile β-lactam, and the hydrolysis rate of the test β-lactam relative to that of nitrocefin was the relative hydrolysis rate. In the β-lactamase inhibition studies, 0.1, 1.0, 10, 100, or 1,000 μmol of BMY-28142 (or other β-lactam) was added to the nitrocefin–β-lactamase reaction mixture, and the hydrolysis rates were calculated as above. The inhibition studies were also confirmed by a previously described method based on a

TABLE 2. Susceptibility to BMY-28142 and related compounds of β-lactamase-positive staphylococci and gram-negative bacteria resistant (MIC ≥ 16 μg/ml) to one or more third-generation cephalosphorins; comparisons with known susceptible strains

Organism	Susceptibility[a]	BMY-28142		Mean MIC differences[b] of:				Cefoperazone
		No.	MIC_{90}	HR 810	Moxalactam	Ceftazidime	Cefotaxime	
Enterobacteriaceae	R	101	4.0	+0.8	+2.7	+3.1	+4.9	+7.2
	S	225	0.13	+0.8	+2.6	+2.5	+2.5	+3.6
Nonfermentative	R	110	16	+0.7	+2.5	−0.6	+2.7	+1.9
gram-negative	S	17	2.0	+0.6	+2.7	+0.2	+2.7	+2.2
bacteria staphylococci	R	46	128	−1.7	+2.5	+2.1	+0.2	−0.1
	S	52	2.0	−1.5	+2.4	+2.0	−0.9	−0.6

[a] Susceptibility of gram-negative bacteria to third-generation cepholosporins. R, resistant to cefoperazone, cefotaxime, moxalactam, or all three; S, susceptible to all three agents. For staphylococci: R, β-lactamase positive; S, β-lactamase negative.
[b] Mean MIC difference from that of BMY-28142 expressed as $\log_2$ concentration.

TABLE 3. Comparative β-lactamase hydrolysis rates of BMY-28142 with those of five other β-lactams

Organism (β-lactamase type)	β-lactamase hydrolysis rate of Nitrocefin (μmol/min)	RHR (%) compared with nitrocefin[a] of:				
		BMY-28142	HR 810	Ceftazidime	Cefoperazone	Cefotaxime
Bacillus cereus (commercial)[b]	33.8	0.8	2.0	0.1	6.7	1.3
Enterobacter cloacae (P99)	51.8	<0.1	<0.1	<0.1	1.2	<0.1
Escherichia coli (OXA1)	7.7	4.8	2.8	4.1	3.1	<1.0
E. coli (OXA3)	3.9	2.1	4.2	2.4	12.5	5.2
E. coli (TEM1)	45.7	0.7	1.1	0.3	24.3	0.2
E. coli (TEM2)	69.8	0.4	1.3	0.2	23.2	0.4
Klebsiella oxytoca (K1)	73.1	1.3	2.4	0.2	1.6	3.1
K. pneumoniae (K14)	48.4	2.0	2.8	0.5	1.0	3.2
P. aeruginosa (CARB1) (PSE-4, plasmid pGM19)	50.2	<1.0	1.0	<1.0	<1.0	4.0
P. aeruginosa (CARB2) (PSE-1, plasmid RPL11)	34.6	<1.0	1.0	<1.0	6.1	<1.0

[a] RHR, Relative hydrolysis rate compared with that of nitrocefin expressed as a value of 100%.
[b] Obtained from BBL Microbiology Systems.

centrifugal fast analyzer (CentrifiChem 400; Union Carbide Corp., Tarrytown, N.Y.) (3, 5).

RESULTS

The MICs of BMY-28142 and three of the comparison drugs for 50 and 90% of each speices tested (MIC_{50} and MIC_{90}, respectively) are shown in Table 1. BMY-28142 exhibited the best activity against the *Enterobacteriaceae*, with an overall MIC_{90} of 1.0 μg/ml and mean MICs of 0.7, 2.4, 2.6, 3.5, and 4.8 $\log_2$ concentrations less than those of HR 810, moxalactam, ceftazidime, cefotaxime, and cefoperazone, respectively. The BMY-28142 MIC_{90} for pseudomonads, in general, was 16 μg/ml; the MIC_{90} for *P. aeruginosa* was 8.0 μg/ml. The mean MICs of BMY-28142 for pseudomonads were 1 and 3 $\log_2$ concentrations lower than those of HR 810 and cefotaxime, respectively, but they were 1 $\log_2$ concentration greater than those of ceftazidime. Against staphylococci, BMY-28142 activity was comparable to those of cefotaxime and cefoperazone, all of which had significantly greater activity than did moxalactam and ceftazidime but nearly fourfold less activity than did HR 810. BMY-28142 demonstrated excellent activity against nonenterococcal streptococci, with MIC_{90}s of 0.015 to 0.5 μg/ml for the various species. The mean MICs of BMY-28142 were within 1 $\log_2$ concentration of those of cefotaxime and HR 810 but were 3 $\log_2$ concentrations less than those of ceftazidime and moxalactam. Strains of *Streptococcus faecalis* were consistently resistant to BMY-28142 (MIC_{90}, 64 μg/ml); cefotaxime and especially HR 810 were more active. Against *Haemophilus influenzae* and the pathogenic *Neisseria* spp., BMY-28142 had marked activity, with MIC_{90}s ranging from ≤0.008 to 0.06 μg/ml. Because of the large number of off-the-scale endpoints with all of the drugs, no valid comparison of activity could be made.

When the gram-negative organisms were divided into resistant and susceptible categories based on resistance (MIC ≥ 16 μg/ml) to one or more of the third-generation drugs (cefotaxime, cefoperazone, and moxalactam) versus susceptibility to all three drugs, the MIC differences between BMY-28142 and the third-generation drugs was most apparent with the *Enterobacteriaceae* (Table 2). The differences among the resistant population were twice those of the susceptible population for cefoperazone and cefotaxime. Among the staphylococci, the differences in MIC betweenBMY-28142 and the other test drugs were not influenced appreciably by β-lactamase production (Table 2).

Of 106 isolates tested with BMY-28142 by both agar dilution and broth microdilution methods, 58% had identical results by the two methods, and all but three (97.2%) had the same (±1) dilution endpoints. Three isolates yielded results

TABLE 4. Inhibition of β-lactamase hydrolysis of nitrocefin by equimolar concentrations of BMY-28142 and six comparison β-lactams[a]

Organism (β-lactamase type)	Nitrocefin RHR (%) combined with[b]:						
	BMY28142	HR 810	Cefoperazone	Cefotaxime	SCH-29482	Sulbactam	Dicloxacillin
Bacillus cereus (commercial)	100	100	<1	100	100	24	100
Enterobacter cloacae (P99)	99	83	<1	<1	<1	30	<1
Escherichia coli (TEM1)	100	100	76	100	26	<1	22
E. coli (TEM2)	100	100	75	100	30	1	20
E. coli (OXA1)	94	100	50	<1	<1	<1	<1
E. coli (OXA3)	100	57	41	<1	<1	<1	<1
Klebsiella oxytoca (K1)	100	100	<1	100	85	62	85
K. oxytoca (K14)	100	100	5	100	89	69	84
P. aeruginosa (CARB1) (PSE-4, plasmid pGM19)	100	100	<1	100	100	<1	46
P. aeruginosa (CARB2) (PSE-1, plasmid RPL11)	100	100	<1	100	100	<1	39

[a] Reference β-lactamase-labile substrate (nitrocefin) concentration was 10^{-4} M in 0.05 M phosphate buffer (ph 7) combined with the inhibiting β-lactam.
[b] RHR, relative hydrolysis rate compared with that of notricefin and added β-lactamase expressed as a value of 100%.

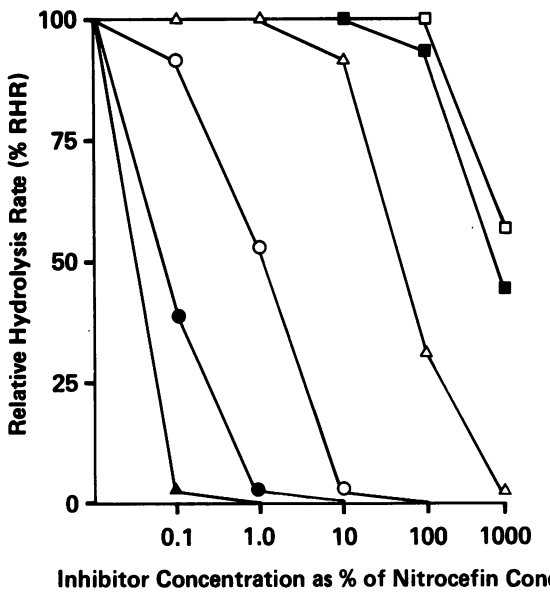

FIG. 1. β-Lactamase inhibition profiles of BMY-28142 and five other β-lactams with a type I (P99) β-lactamase derived from *E. cloacae*. Base-line hydrolysis rates were those of nitrocefin at a concentration of 10^{-4} M. Five concentrations of each β-lactam were tested: dicloxacillin (▲), cefotaxime (●), SCH-29482 (○), sulbactam (△), HR 810 (■), and BMY-28142 (□). (RHR, relative hydrolysis rate).

that differed by two doubling dilutions. On balance, the agar MICs averaged 0.15 $\log_2$ concentrations higher than the broth MICs, an insignificant difference.

The overall susceptibility pattern of BMY-28142 in this study was most like that of HR 810. When the MICs of both drugs with 695 organisms were qualitatively categorized as susceptible (MIC ≤ 8 μg/ml), intermediate (MIC, 16 μg/ml), and resistant (MIC ≥ 32 μg/ml), 92.4% of the isolates fell into the same category for both drugs. Of the remaining 7.6% of the isolates, 4.3% represented minor differences (susceptible-intermediate or resistant-intermediate), and 3.3% represented major differences (susceptible-resistant). Nearly half of the discrepancies were accounted for by *S. faecalis*, which was generally resistant to BMY-28142 and susceptible or intermediate to HR 810. If this species is dropped from the comparison, the category correlations between these two drugs becomes 95.6% with 3.1% minor differences and 1.3% major differences.

The β-lactamase stability of BMY-28142 was tested against 10 common bacterial β-lactamases in parallel with the other comparative drugs (Table 3). It was very enzyme stable, with relative hydrolysis rates comparable to those of HR 180, ceftazidime, cefotaxime, and moxalactam. Cefoperazone exhibited significantly less stability against OXA 3, TEM 1, and TEM 2 β-lactamases.

BMY-28142 inhibition of the β-lactamase hydrolysis of nitrocefin was tested with the same β-lactamases and compared with six other β-lactam drugs with various inhibition profiles (Table 4). BMY-28142 demonstrated virtually no β-lactamase affinity and again was most like HR 810. The differences in inhibitory activity and affinity between BMY-28142 and the other drugs are graphically displayed in Fig. 1 with a type I (P99) β-lactamase.

DISCUSSION

BMY-28142 joins the growing list of newer cephalosporins showing an improved spectrum of in vitro antimicrobial activity. Of the five comparison drugs studied, the activity and spectrum of BMY-28142 were most similar to those of HR 810, but some differences were noted. Generally, BMY-28142 was slightly more active than HR 810 against gram-negative bacteria and less active against gram-positive bacteria. It was the most active agent tested against *Enterobacteriaceae* and second only to ceftazidime against pseudomonads. It was comparable to cefotaxime against staphylococci and nonenterococcal streptococci, but it was the least active of the drugs that could be compared against the enterococci. Furthermore, this excellent activity against gram-negative bacteria was observed against a collection of bacteria (particularly *Enterobacteriaceae*) resistant to third-generation cephalosporins (Table 2). Of note are the greater MIC differences between BMY-28142 and some third-generation cephalosporins (particularly cefoperazone and cefotaxime) with resistant strains of *Enterobacteriaceae* compared with susceptible strains.

The excellent spectrum of BMY-28142, its virtual complete resistance to the action of common β-lactamases, and its low affinity for these enzymes are characteristics that make this a promising drug. Studies on its toxicology and pharmacokinetics in humans are required to further analyze the significance of these in vitro data.

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