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In Vitro Activity of BRL 36650, a New Semisynthetic Penicillin

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BRL 36650 [sodium 6β-{D-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-(3,4-dihydroxyphenyl) acetamido}-6α-formamido-penicillinate] is a new semisynthetic penicillin. It was tested in vitro for activity against 884 organisms cultured from blood specimens of cancer patients. BRL 36650 had broad-spectrum activity against the gram-negative bacilli tested but had no gram-positive activity. The MIC against 90% of the *Pseudomonas aeruginosa* isolates was 3.12 μg/ml. The activity of BRL 36650 was superior to that of piperacillin, comparable or slightly inferior to that of aztreonam and ceftazidime, and lower than that of imipenem and amifloxacin. BRL 36650 should prove useful for the management of gram-negative bacillary infections, including those caused by *P. aeruginosa*.

Infections in neutropenic cancer patients have traditionally been treated with a combination of a β-lactam and an aminoglycoside (2). This combination has become less useful because of an increasing resistance to antimicrobial agents and because of toxicity (5, 7). There is a recent trend toward the use of monotherapy and double β-lactam combinations in the treatment of infections in this group of patients (6). Consequently, there is a continuing search for new broad-spectrum β-lactam antibiotics.

BRL 36650 [sodium 6β-{D-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino] - 2 - (3,4 - dihydroxyphenyl)acetamido}-6α-formamido-penicillinate] is a new synthetic penicillin in which a formamido group has been introduced into the 6α position of the penicillin nucleus. This has been shown to confer β-lactamase stability without compromising antibacterial activity (1). BRL 36650 has been shown to be highly active against aerobic gram-negative bacilli, including *Pseudomonas aeruginosa*, an important cause of morbidity and mortality in cancer patients (4). However, it has minimal activity against gram-positive cocci. We evaluated the activity of BRL 36650 against 884 clinical isolates from cancer patients and compared its activity with that of seven other antibiotics.

MATERIALS AND METHODS

BRL 36650 was supplied by Beecham Pharmaceuticals Research Division, Surrey, England. Amifloxacin was supplied by Sterling Winthrop Research Institute, Rensselaer, N.Y. Amikacin was supplied by Bristol Laboratories, Syracuse, N.Y. Imipenem was supplied by Merck Sharp & Dohme, Rahway, N.J. Aztreonam was supplied by E. R. Squibb & Sons, Princeton, N.J. Ceftazidime was supplied by Glaxo Research Ltd., Research Triangle Park, N.C. Piperacillin was supplied by Lederle Laboratories, Pearl River, N.Y., and trimethoprim-sulfamethoxazole was supplied by Hoffman-LaRoche Inc., Nutley, N.J.

A total of 652 isolates of gram-negative bacilli and 232 isolates of gram-positive cocci were tested. All organisms were isolated from blood cultures obtained from cancer patients at this institution during the past 5 years. All isolates were maintained in stock by lyophilization or ultrafreezing methods. Organisms were tested in duplicate simultaneously by two investigators (J.F.H. and M.A.). *Staphylococcus aureus* isolates were considered penicillin G susceptible on the basis of an MIC of <0.1 μg/ml, methicillin susceptible on the basis of an MIC of ≤3.12 μg/ml, and methicillin resistant on the basis of an MIC ≥12.5 μg/ml.

Organisms were inoculated into broth cultures and incubated at 37°C for 18 h. Appropriate dilutions were made so that the final concentration of organisms was 10^5 CFU/ml. The concentration and purity of all isolates were confirmed by plate counting.

The test medium used was Mueller-Hinton broth (MH; Difco Laboratories, Detroit, Mich.) for all organisms except group A and group G streptococci which were tested in tryptose-phosphate broth, CDC-JK diphtheroids which were tested in brain heart infusion (BHI) and 5% rabbit serum, and staphylococci which were tested in MH supplemented with 5% NaCl, Mg^{2+} (25 mg/liter), and Ca^{2+} (50 mg/liter). Amikacin was prepared in cation-supplemented MH for the testing of *P. aeruginosa*.

Antibiotic concentrations were prepared manually with serial twofold dilutions ranging from 50 to 0.025 μg/ml and dispensed automatically by using an MIC 2000 dispenser (Dynatech Laboratories, Inc., Alexandria, Va.) for the comparative studies of the eight antibiotics. The plates were automatically inoculated with 0.1 ml of antibiotic dilution. Each organism was tested against all antibiotic agents simultaneously. *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 were included as controls for each procedure.

The effect of inoculum size was determined with concentrations of 10^4, 10^5, 10^6, and 10^7 CFU of 10 strains each of *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *P. aeruginosa* per ml. The same 10 strains of each organism were also used for studies on the effect of pH variation and different media on the MICs. The pH was adjusted with HCl or NaOH.

The MIC was defined as the lowest concentration of drug which suppressed visible growth after incubation at 37°C for 18 to 24 h for gram-negative bacilli and gram-positive cocci, except for streptococci which were incubated at 37°C for 18 to 24 h in a CO_2 incubator. The MICs for *Staphylococcus* spp. were determined at 24 and 48 h and were not found to be significantly different.

Because the MICs obtained in our study were severalfold

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TABLE 1. Comparative in vitro activity of antibiotics against gram-negative bacilli

Organism (no.)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
Acinetobacter anitratus (40)	BRL 36650	3.12	6.25	1.56–12.5
	Piperacillin	12.5	25	3.12–>50
	Amikacin	1.56	6.25	0.39–12.5
	Aztreonam	25	50	3.12–50
	Ceftazidime	6.25	12.5	1.56–25
	Imipenem	0.20	0.39	0.10–0.78
	Amifloxacin	0.39	0.78	0.20–1.56
	TMP-SMX[b]	0.25 + 4.75	1 + 19	0.06 + 1.18–>(4 + 76)
Acinetobacter lwoffii (40)	BRL 36650	3.12	6.25	0.78–12.5
	Piperacillin	3.12	12.5	1.56–50
	Amikacin	0.39	3.12	0.05–25
	Aztreonam	6.25	25	0.20–50
	Ceftazidime	3.12	6.25	0.20–12.5
	Imipenem	0.10	0.20	≤0.025–3.12
	Amifloxacin	0.39	0.78	≤0.025–3.12
	TMP-SMX	0.125 + 2.37	0.25 + 4.75	0.015 + 0.30–2 + 38
Citrobacter diversus (20)	BRL 36650	3.12	6.25	0.78–6.25
	Piperacillin	6.25	25	6.25–>50
	Amikacin	1.56	3.12	0.78–6.25
	Aztreonam	0.05	0.78	≤0.025–12.5
	Ceftazidime	0.20	0.78	0.10–25
	Imipenem	0.20	0.39	0.10–0.39
	Amifloxacin	0.10	0.10	0.05–0.39
	TMP-SMX	0.03 + 0.59	0.125 + 2.37	0.03 + 0.59–1 + 19
Citrobacter freundii (30)	BRL 36650	3.12	6.25	0.78–12.5
	Piperacillin	25	>50	0.39–>50
	Amikacin	0.78	1.56	0.39–3.12
	Aztreonam	0.20	25	≤0.025–50
	Ceftazidime	1.56	>50	0.10–>50
	Imipenem	0.39	0.39	0.20–0.78
	Amifloxacin	0.10	0.39	≤0.025–0.78
	TMP-SMX	0.25 + 4.75	>(4 + 76)	0.015 + 0.30–>(4 + 76)
Enterobacter aerogenes (20)	BRL 36650	3.12	6.25	0.78–12.5
	Piperacillin	3.12	>50	1.56–>50
	Amikacin	1.56	1.56	0.78–3.12
	Aztreonam	0.10	25	0.05–50
	Ceftazidime	0.78	>50	0.20–>50
	Imipenem	0.39	1.56	0.20–3.12
	Amifloxacin	0.20	0.39	0.05–0.78
	TMP-SMX	0.06 + 1.18	4 + 76	0.03 + 0.59–>(4 + 76)
Enterobacter agglomerans (13)	BRL 36650	3.12	6.25	0.78–6.25
	Piperacillin	1.56	6.25	0.78–6.25
	Amikacin	0.78	1.56	0.20–1.56
	Aztreonam	0.05	0.39	≤0.025–0.78
	Ceftazidime	0.20	0.78	0.05–1.56
	Imipenem	0.39	0.39	0.20–0.39
	Amifloxacin	0.05	0.20	0.05–0.39
	TMP-SMX	0.015 + 0.30	0.06 + 1.18	≤(0.0037 + 0.08)–0.5 + 9.5
Enterobacter cloacae (50)	BRL 36650	3.12	6.25	1.56–>50
	Piperacillin	6.25	>50	0.78–>50
	Amikacin	0.78	1.56	0.39–3.12
	Aztreonam	0.20	25	≤0.025–>50
	Ceftazidime	0.78	>50	0.10–>50
	Imipenem	0.39	0.78	0.10–3.12
	Amifloxacin	0.10	0.39	≤0.025–0.39
	TMP-SMX	0.06 + 1.18	0.25 + 4.75	0.03 + 0.59–>(4 + 76)
Escherichia coli (100)	BRL 36650	1.56	3.12	0.39–25
	Piperacillin	1.56	>50	0.39–>50
	Amikacin	1.56	3.12	0.39–50
	Aztreonam	0.05	0.10	≤0.025–0.39
	Ceftazidime	0.20	0.39	0.05–1.56

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TABLE 1—*Continued*

Organism (no.)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
	Imipenem	0.20	0.20	≤0.025–3.12
	Amifloxacin	0.05	0.10	≤0.025–6.25
	TMP-SMX	0.06 + 1.18	4 + 76	0.015 + 0.30–>(4 + 76)
Klebsiella oxytoca (25)	BRL 36650	3.12	6.25	1.56–6.25
	Piperacillin	6.25	>50	3.12–>50
	Amikacin	0.78	1.56	0.39–3.12
	Aztreonam	0.20	12.5	≤0.025–>50
	Ceftazidime	0.20	0.78	0.10–6.25
	Imipenem	0.20	0.39	0.20–0.39
	Amifloxacin	0.10	0.10	≤0.025–0.39
	TMP-SMX	0.06 + 1.18	0.125 + 2.37	0.03 + 0.59–>(4 + 76)
Klebsiella pneumoniae (100)	BRL 36650	1.56	6.25	0.39–12.5
	Piperacillin	6.25	>50	1.56–>50
	Amikacin	0.78	1.56	0.39–6.25
	Aztreonam	0.05	0.20	≤0.025–25
	Ceftazidime	0.20	0.78	0.10–12.5
	Imipenem	0.20	0.39	0.10–0.78
	Amifloxacin	0.20	0.78	0.05–1.56
	TMP-SMX	0.06 + 1.18	>(4 + 76)	0.03 + 0.59–>(4 + 76)
Pseudomonas aeruginosa (100)	BRL 36650	3.12	3.12	0.78–25
	Piperacillin	6.25	>50	1.56–>50
	Amikacin	6.25	25	0.78–>50
	Aztreonam	6.25	50	1.56–>50
	Ceftazidime	1.56	50	0.39–>50
	Imipenem	1.56	3.12	0.78–>50
	Amifloxacin	0.78	3.12	0.39–6.25
	TMP-SMX	>(4 + 76)	>(4 + 76)	2 + 38–>(4 + 76)
Proteus mirabilis (50)	BRL 36650	1.56	3.12	0.78–12.5
	Piperacillin	0.39	0.78	0.20–25
	Amikacin	3.12	6.25	0.78–12.5
	Aztreonam	≤0.025	≤0.025	≤0.025
	Ceftazidime	0.10	0.10	≤0.025–0.20
	Imipenem	1.56	3.12	0.10–12.5
	Amifloxacin	0.20	0.20	0.10–0.39
	TMP-SMX	0.06 + 1.18	0.5 + 9.5	0.03 + 0.59–>(4 + 76)
Proteus vulgaris (14)	BRL 36650	1.56	6.25	0.78–12.5
	Piperacillin	1.56	>50	0.20–>50
	Amikacin	1.56	3.12	0.39–3.12
	Aztreonam	≤0.025	≤0.025	≤0.025–0.78
	Ceftazidime	0.05	0.20	0.05–12.5
	Imipenem	1.56	3.12	0.39–6.25
	Amifloxacin	0.10	0.20	0.10–0.20
	TMP-SMX	0.06 + 1.18	0.125 + 2.37	0.06 + 1.18–0.125 + 2.37
Serratia marcescens (50)	BRL 36650	3.12	3.12	0.78–3.12
	Piperacillin	3.12	50	0.78–>50
	Amikacin	1.56	3.12	0.78–6.25
	Aztreonam	0.10	0.39	≤0.025–6.25
	Ceftazidime	0.20	0.78	0.10–3.12
	Imipenem	0.78	0.78	0.10–6.25
	Amifloxacin	0.20	0.39	0.10–6.25
	TMP-SMX	0.25 + 4.75	0.5 + 9.5	0.125 + 2.37–1 + 19

[a] 50% and 90%, MIC of antibiotic that inhibited 50 and 90%, respectively, of the isolates.
[b] TMP-SMX, Trimethoprim-sulfamethoxazole.

higher than MICs previously reported (1), we obtained fresh BRL 36650 antibiotic powder (Beecham Pharmaceuticals) and retested 100 gram-negative isolates (20 *P. aeruginosa*, 20 *Serratia marcescens*, 20 *K. pneumoniae*, 20 *E. coli*, and 20 *E. cloacae*) in a manner identical to that of the original study. The original and the fresh BRL 36650 powder were tested on the same microtiter plate for comparison, and both antibiotics were tested in MH obtained from Difco and in MH obtained from BBL Microbiology Systems, Cockeysville, Md., as suggested by Beecham Laboratories. The original BRL 36650 was obtained in October 1984 and stored at 0 to 5°C in a dessicator.

TABLE 2. Effect of medium variation on activity of BRL 36650

Organism	MIC (μg/ml)[a] of BRL 36650 in various media[b]					
	MH	MH/HS	BHI	TSB	NB	IsoTest
Escherichia coli	3.12	0.10	0.78	6.25	12.5	12.5
Klebsiella pneumoniae	3.12	0.05	1.56	6.25	12.5	6.25
Enterobacter cloacae	25	6.25	25	25	25	>50
Pseudomonas aeruginosa	6.25	3.12	25	25	25	6.25

[a] Expressed as the concentration needed for 10 isolates tested.
[b] MH, Mueller-Hinton broth; MH/HS, 50% human serum in MH; BHI, brain heart infusion; TSB, tryptose soya broth; NB, nutrient broth; IsoTest, Iso-Sensitest broth.

RESULTS

BRL 36650 showed no activity against most of the 232 gram-positive isolates tested, with the exception of group G beta-hemolytic streptococci (MIC for 90% of the isolates tested [MIC_{90}], 6.25 μg/ml; data not shown). Gram-positive organisms tested included penicillin-susceptible, methicillin-susceptible, and methicillin-resistant *S. aureus*; coagulase-negative staphylococci, streptococci, enterococci; JK diphtheroids; and *Listeria* spp. The concentrations of each antibiotic required to inhibit 50 and 90% of the 652 gram-negative isolates tested are shown in Table 1.

BRL 36650 was more active than piperacillin against all the gram-negative bacilli. It was extremely active against all *P. aeruginosa*, with an MIC_{90} of 3.12 μg/ml. This activity was equivalent to that of imipenem and amifloxacin and superior to that of all other antibiotics tested. The activity of BRL 36650 against members of the family *Enterobacteriaceae* was uniformly good, with MIC_{90}s in the range of 3.12 to 6.25 μg/ml. *E. cloacae*, *Enterobacter aerogenes*, and *Citrobacter freundii* isolates were more susceptible to BRL 36650 than they were to most of the other antibiotics tested, with the exception of imipenem and amifloxacin. Although the activity of BRL 36650 was good against other members of the *Enterobacteriaceae*, the drug was two- to fourfold less active than aztreonam, ceftazidime, imipenem, and amifloxacin. BRL 36650 was more active than most other antibiotics (except imipenem and amifloxacin) against *Acinetobacter* spp., with an MIC_{90} of 6.25 μg/ml.

On retesting 100 gram-negative bacilli with fresh BRL 36650 powder and using the original BRL 36650 on the microtiter plate for comparison, we obtained MIC results identical to those obtained in the original study. We also used MH obtained from BBL, as suggested by Beecham, comparing results with those on MH obtained from Difco, with both the original and fresh BRL 36650. Again, there was no difference in the MIC_{90} obtained.

The effect of inoculum size was determined for 10 strains each of *E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa*. The MICs for *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were essentially the same for inoculum sizes of 10^4, 10^5, and 10^6 CFU/ml. However, when the inoculum was increased to 10^7 CFU/ml, the MICs increased to >50 μg/ml. For *E. cloacae*, the MIC gradually increased from 1.56 μg/ml for an inoculum of 10^4 CFU/ml to >50 μg/ml when the inoculum was 10^7/ml. The pH variation of the media did not significantly alter the activity of BRL 36650 against *E. cloacae* or *P. aeruginosa*. The activity of BRL 36650 for *E. coli* and *K. pneumoniae* was minimally enhanced at an acid pH.

The same 10 strains of each organism were tested in MH, 50% human serum in MH (MH/HS), BHI, tryptose soy broth, nutrient broth, and Iso-Sensitest broth (IsoTest) to examine the effects of medium variation on the MICs (Table 2). When TSB, NB, and IsoTest were used as the test media, the MICs were found to be four- to eightfold higher than those of the same organisms tested in MH. The activity of BRL 36650 against *E. coli* and *K. pneumoniae* was enhanced when tested in BHI compared with its activity in MH. However, the activity was unchanged for *E. cloacae* and reduced for *P. aeruginosa* when tested in BHI. The addition of 50% HS to MH resulted in a markedly enhanced increase in the activity of BRL 36650 against *K. pneumoniae* and *E. coli*, a moderate increase in the activity against *E. cloacae*, and no differences in the activity against *P. aeruginosa*.

DISCUSSION

The antibacterial activity of BRL 36650 appears limited to gram-negative organisms. It has superior activity against *P. aeruginosa* and good activity against members of the *Enterobacteriaceae* and *Acinetobacter* spp. However, the activity of BRL 36650 against organisms from our institution appeared inferior to that which was reported by Basker et al. (1). This difference in activity could be due to a number of factors. First, the organisms obtained from cancer patients in our institution may have been more resistant in general because of the greater exposure of the organisms to the newer broad-spectrum antibiotics. The activity of the other antibiotics used for comparison was equivalent to that previously reported from this institution (3). Secondly, the methodology differed between the two studies. Basker et al. used an inoculum size of 5×10^4 CFU/ml, and the medium used was Diagnostic Sensitivity Test agar. Basker et al. noted a twofold difference in MICs when broth was used instead of agar to perform the MIC testing. Their MIC results were 8- to 16-fold lower than our results for the members of the *Enterobacteriaceae*; however, MICs were similar to our results for *P. aeruginosa* (1).

BRL 36650 was found to be 35% bound to HS protein by Basker et al., who also noted the activity of the drug was either unaffected or reduced two- to fourfold by the presence of 50% HS (1). This again is in contrast to our findings which revealed an enhanced activity of BRL 36650, in the presence of 50% HS, especially against *E. coli* and *K. pneumoniae*. Basker et al. found no significant change in the activity of BRL 36650 when tested in various different agar media, except against three strains of *P. aeruginosa*. Reduced activity was found when these three strains were tested in MH agar. The contrast between the findings of Basker et al. and our findings for various media can be explained by differences in methodology. Basker et al. performed their tests in agar media; ours were performed in broth media. We tested 10 strains of four organisms in different media, whereas Basker et al. tested single strains.

In this study BRL 36650 was found to have a broad spectrum of activity against gram-negative bacilli including members of the *Enterobacteriaceae*, although its activity was not as good as that of ceftazidime, imipenem, or the quinolone amifloxacin against some common pathogens. BRL 36650, however, had superior activity against *P. aeruginosa* and *Enterobacter* spp. compared with other antibiotics tested. Its activity was far superior to that of piperacillin, the other penicillin tested; however, it had no gram-positive activity. This new agent deserves to be evaluated further in clinical trials.

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