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NOTES

In Vitro Activity of BRL 17421 Against *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Branhamella catarrhalis*

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BRL 17421, a novel β-lactam antibiotic, was tested in vitro against fastidious gram-negative bacteria and compared with amoxicillin and amoxicillin plus clavulanic acid. The compound showed good activity against *Haemophilus influenzae* (range of minimal inhibitory concentrations, 0.2 to 1 μg/ml), *Neisseria gonorrhoeae* (0.007 to 0.5 μg/ml), and *Branhamella catarrhalis* (0.03 to 0.1 μg/ml). BRL 17421 exhibited excellent stability against the TEM-type β-lactamase of *H. influenzae* and *N. gonorrhoeae*, and its activity was little affected by inoculum size. Minimal lethal concentrations of BRL 17421 for 10^7 colony-forming units of *H. influenzae* ranged between 0.5 and 4 μg/ml.

BRL 17421, 6β-(2-carboxy-2-thien-3-yl acetamido)-6α-methoxypenicillanic acid, disodium salt, is a novel β-lactam antibiotic with good activity against most *Enterobacteriaceae*, but not against gram-positive bacteria. The compound is very resistant to bacterial β-lactamases. After parenteral dosage, serum half-life is 5 h, with peak serum concentrations of 70 and 48 μg/ml after a single intramuscular injection of 1,000 and 500 mg, respectively (3). In this study, the in vitro antimicrobial activity of BRL 17421 was compared with the activity of amoxicillin alone and in combination with clavulanic acid, against four fastidious gram-negative bacterial species.

The antimicrobial agents were obtained from Beecham Pharmaceuticals (Bristol, Tenn.). Amoxicillin was tested in the presence of clavulanic acid at a ratio of 2 to 1. The following strains were studied: 25 β-lactamase-producing and 25 β-lactamase-negative *Haemophilus influenzae*; 28 β-lactamase-producing and 25 β-lactamase-negative *Neisseria gonorrhoeae*; 10 *Branhamella catarrhalis* (including 5 β-lactamase-positive strains).

Minimal inhibitory concentrations (MICs) were determined with an agar dilution technique on Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) supplemented with 2% Fildes enrichment (Difco) for *H. influenzae* and GC agar base (Difco) supplemented with 1% hemoglobin (Difco) and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) for the other species. The final inoculum was 10^5 colony-forming units (CFU), except for *H. influenzae*, which was tested with 10^4 and 10^6 CFU. All plates were incubated in a 5% CO_2 incubator at 35°C. MICs were read after 24 h (defined as the lowest concentration of antimicrobial agent completely inhibiting bacterial growth).

Minimal lethal concentrations (MLCs) were determined in Mueller-Hinton broth (Difco) supplemented with 5% Fildes enrichment. Tubes containing approximately 10^7 CFU/ml were incubated at 37°C in a 5% CO_2 incubator. After 18 h, the MIC was registered, and 0.1 ml from the tubes showing no turbidity was plated onto GC agar base with 1% hemoglobin and 1% IsoVitaleX. The MLC was the lowest concentration killing 99.9% of the initial inoculum.

The results are shown in Table 1. The MICs for *H. influenzae* were obtained with 10^4 CFU. BRL 17421 was at least as active as amoxicillin against the β-lactamase-negative strains, but was more active against the β-lactamase-producing strains. The compound displayed a high stability against the TEM-type β-lactamase of *H. influenzae* and *N. gonorrhoeae*, with an average increase of 1 to 2 dilution steps for the MICs of the β-lactamase-producing *N. gonorrhoeae* strains as compared with the penicillin-sensitive strains. The same phenomenon occurred with *B. catarrhalis*, which produces a particular type of β-lactamase. The MICs of BRL 17421 for *H. influenzae* were little affected when the inoculum size was increased from 10^4

TABLE 1. MICs of BRL 17421, amoxicillin, and amoxicillin plus clavulanic acid for three bacterial species

Species[a]	No. of strains	MIC_{50} (range) (μg/ml) for:		
		BRL 17421	Amoxicillin	Amoxicillin plus clavulanic acid
H. influenzae β+	25	0.2 (0.2–1)	2 (1–16)	0.5 (0.2–1)
β−	25	0.5 (0.2–1)	0.5 (0.2–0.5)	Not tested
N. gonorrhoeae β+	28	0.1 (0.03–0.5)	16 (4–16)	0.1 (0.01–2)
β−	25	0.03 (0.007–0.5)	0.06 (0.007–2)	Not tested
B. catarrhalis	10	0.06 (0.03–0.1)	0.06 (0.01–1)	0.03 (0.01–0.06)

[a] Inoculum, 10^4 CFU per spot.

to 10^6 CFU with respective MIC_{50}'s of 0.2 and 0.5 μg/ml for the β-lactamase-producing strains. The latter inoculum corresponds better to the number of bacteria found in cerebrospinal fluid in *H. influenzae* meningitis (1). The MICs and MLCs for *H. influenzae* with an inoculum of 10^7 CFU/ml are given in Table 2. In general MLC values for individual strains increased with not more than one dilution, with the exception of three strains, including one β-lactamase producer, which gave MLCs four to eight times higher than the MIC values in broth.

The MICs of amoxicillin were significantly reduced in the presence of clavulanic acid for the β-lactamase-producing strains, as reported earlier for *H. influenzae* (4) and *N. gonorrhoeae* (2).

In conclusion, BRL 17421 was highly active against the strains tested, including all β-lactamase-positive strains. There was no marked inoculum effect with the β-lactamase-producing strains, illustrating the stability of BRL 17421 to β-lactamases. The bactericidal activity against large numbers of *H. influenzae* was good. In view of the favorable pharmacokinetic properties and the good in vitro activity, this compound deserves clinical evaluation for the treatment of infections due to *N. gonorrhoeae* and *H. influenzae*, including those caused by β-lactamase-producing strains.

TABLE 2. MICs (broth dilution test) and MLCs of BRL 17421 against *H. influenzae*[a]

β-lactamase production	No. of strains	MIC_{50} (range) (μg/ml)	MLC_{50} (range) (μg/ml)
−	8	0.5 (0.2–0.5)	1 (0.5–2)
+	10	0.5 (0.2–0.5)	0.5 (0.5–4)

[a] Inoculum, 10^7 CFU/ml.

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