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In Vitro Activity of Penicillins Against Anaerobes

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The in vitro susceptibility of 162 anaerobic isolates from clinical material were tested to pencillin G, BL-P1654, and carbenicillin. Penicillin G and BL-P1654 showed good activity against *Bacteroides fragilis*, but only 60% of strains were susceptible to carbenicillin at achievable blood levels (128 μg/ml).

Bacteroides fragilis occupies a preeminent position in anaerobic infections because of its frequency of isolation and its resistance to commonly used antimicrobial agents (4). At present, the antimicrobial agents recommended for treating infections with this organism are limited to clindamycin and chloramphenicol. Serious complications with both of these antimicrobials have led to a search for alternate agents (11).

Several laboratories have assessed the activity of penicillins against *B. fragilis* (6-8). Martin et al. showed that penicillin G was active against 85% of 195 isolates of *B. fragilis* at 25 μm/ml but demonstrated little activity at the 1 μg/ml level (8). Recently, carbenicillin at high concentrations has been shown to inhibit most "Bacteroides" (2). Preliminary in vitro studies indicated that BL-P1654, a new semisynthetic ureidopenicillin, has a spectrum similar to that of carbenicillin (9). This study is a comparison of the in vitro activity of penicillin G, BL-P1654, and carbenicillin against a variety of anaerobes.

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MATERIALS AND METHODS

The test strains included 162 isolates of various anaerobic bacteria cultured from clinical material in the Anaerobic Research Laboratory, Sepulveda VA Hospital, between May 1973 and March 1974. The organisms were isolated utilizing anaerobic chamber techniques and were identified according to the criteria of VPI *Anaerobic Laboratory Manual* (5).

The procedure used for the antimicrobial susceptibility testing was a modified agar dilution method, utilizing a Steer's replicator (10). Briefly, strains were inoculated into supplemented, prereduced, brain-heart infusion broth and incubated anaerobically for 5 to 6 h (for rapid growers) or for 24 h (for slow growers). The inoculum was diluted to deliver approximately 10^4 colony-forming units to the surface of freshly prepared 5% laked sheep blood plates supplemented with vitamin K_1 (10 μg/ml) and two-fold serial dilutions of the three antibiotics. Inoculations were carried out in the anaerobic chamber. The plates were incubated for 48 h at 37 C in the chamber with an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. The minimal inhibitory concentration was defined as the lowest concentration of antibiotics which showed no growth, a barely visible haze or a single colony (3).

The anaerobic bacteria tested were *B. fragilis* (33), *B. melaninogenicus* (5), other bacteroides (4), *Fusobacterium nucleatum* (4), *Fusobacterium species* (7), *Peptococcus* sp. (19), *Peptostreptococcus* sp. (25), *Veillonella* sp. (9), *Eubacterium* sp. (10), nonspore-forming gram-positive rods (4), *Propionobacterium* sp. (10), *Clostridium perfringens* (15), and *Clostridium* sp. (17).

RESULTS

Penicillin G and BL-P1654 demonstrated better activity than carbenicillin against 33 isolates of *B. fragilis* (Fig. 1). Penicillin G inhibited 91% of strains at 32 μm/ml. BL-P1654 inhibited 88% of strains at this level. In contrast carbenicillin inhibited only 60% of the strains at 128 μg/ml. All three drugs exhibited good activity against the 129 anaerobic strains other than *B. fragilis*: at 1 μm/ml 76% were suscepti-

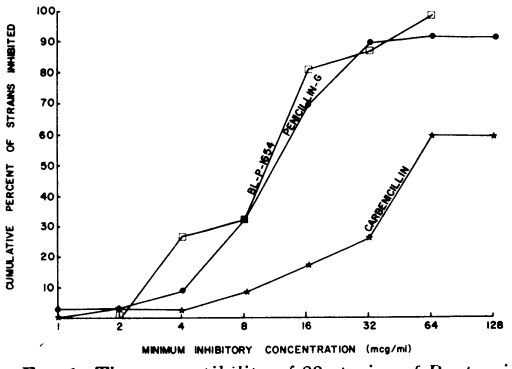

FIG. 1. *The susceptibility of 33 strains of Bacteroides fragilis to penicillin G. BL-P1654 and carbenicillin. Symbols:* ●, *penicillin G;* ★, *Carbenicillin;* □, BL-P1654.

TABLE 1. *Susceptibility of 129 other anerobic bacteria to three penicillins*

Antibiotic	Cumulative % susceptible at MIC (μg/ml)								
	0.1	1.0	2.0	4.0	8.0	16	32	64	128
Penicillin	47	76	86	92	97	98	98	99	99
BL-P1654	38	82	88	91	95	98	98	99	99
Carbenicillin	12	46	64	75	82	85	98	99	99

ble to penicillin G, 82% to BL-P1654, and 46% to carbenicillin (Table 1). At 16 μg 85 to 98% were susceptible to the three agents.

Resistant strains other than *B. fragilis* included an unspeciated *Fusobacterium* which was resistant to all three drugs. One isolate of *F. varium* required 64 μg of penicillin G per ml for inhibition. There was also a strain of *C. septicum* which required 64 μg of BL-P1654 per ml for inhibition and was resistant to 128 μg or greater of carbenicillin per ml. Two additional organisms, a *P. acnes* and a *E. lentum*, were resistant to carbenicillin at 128 μg/ml or greater.

DISCUSSION

Penicillin G susceptibility with conventional dosage is usually defined as an MIC of 1 μg/ml or less. With high parenteral dosage, an MIC of 32 μg/ml or less could be considered acceptable (4). Mean peak BL-P1654 blood levels of 47.5 μg/ml are achieved with 1-g doses; therefore, organisms with MICs of 32 μg/ml or less were considered susceptible (1). Finally, organisms with MICs of 128 μg/ml or less are usually considered susceptible to carbenicillin (8).

The results of this study indicate that BL-P1654 has in vitro activity comparable to penicillin G against anaerobes. Both agents are significantly more active against *B. fragilis* than carbenicillin, even taking account of the superior serum levels achievable with the latter agent. All three penicillins showed generally good activity at easily achievable serum levels against the other 129 anaerobes tested. However, again penicillin G and BL-P1654 were superior to carbenicillin on a weight basis.

The recent suspension of clinical trials with BL-P1654 because of nephrotoxicity precludes evaluation of the agent for anaerobic infection. However, its in vitro activity against *B. fragilis* promises the possibility that other new penicillins may possess good activity against this organism.

Our findings support the published observation that penicillin G in high doses has good in vitro activity against most strains of *B. fragilis* (4). There have been documented treatment failures with high dose penicillin G in patients with anaerobic infections, but the reasons for these failures are not readily apparent (12). The treatment with high dose penicillin G of anaerobic infections involving *B. fragilis* in an animal model should be a prerequisite to well controlled human clinical trials of megadose penicillin G therapy of anaerobic infections involving *B. fragilis*.

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