Comparison of the In Vitro Activity of BL-P1654 with Gentamicin and Carbenicillin Against Pseudomonas aeruginosa

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The in vitro activity of 6-[α-(3 guanylureido)-phenylacetamido]-penicillanic acid (BL-P1654) was evaluated against 117 clinical isolates of Pseudomonas aeruginosa, many of which were known to be resistant to both gentamicin and carbenicillin. BL-P1654 was two to eight times more active than carbenicillin against P. aeruginosa. However, all 28 highly carbenicillin-resistant isolates (minimal inhibitory concentration [MIC] > 500 µg/ml) were also highly resistant to BL-P1654. When an MIC of 32 µg/ml or less and a zone of inhibition of 12 mm or more by a 10-µg disk were used as criteria indicating susceptibility to BL-P1654, the false-resistance rate by the disk test was 10.6% and the false-susceptibility rate was 4.2%. The combination of BL-P1654 and gentamicin was synergistic against 45 of 70 isolates of P. aeruginosa tested, but synergism was demonstrated against only 4 of 24 highly gentamicin-resistant (MIC > 63 µg/ml), 1 of 12 highly BL-P1654-resistant (MIC > 250 µg/ml) isolates, and none of nine isolates highly resistant to both of these antibiotics.

The availability of carbenicillin (carboxybenzylpenicillin) for clinical use has significantly contributed to the therapy of serious infections due to Pseudomonas aeruginosa, since it is a semisynthetic penicillin and is not ototoxic or renal toxic. However, carbenicillin is only moderately active against P. aeruginosa in vitro (4, 12, 14, 16) and large doses are necessary for the therapy of systemic infections (7, 11). Furthermore, increasing numbers of clinical isolates of P. aeruginosa are resistant to carbenicillin (6, 8, 9). A semisynthetic penicillin with a more potent antipseudomonal activity is clearly desirable.

Further chemical modification of the side chain of benzylpenicillin has resulted in a new compound, 6-[α-(3-guanylureido)-phenylacetamido]-penicillanic acid, designated BL-P1654. Preliminary investigations revealed its in vitro effectiveness against Enterobacteriaceae and its marked antipseudomonal activity (3, 13, 17).

In the present investigation, clinical isolates of P. aeruginosa, many of which had been known to be resistant to carbenicillin and/or gentamicin, were studied with the following objectives: (i) the determination of the in vitro activity of BL-P1654 in comparison with gentamicin and carbenicillin; (ii) the correlation of the minimal inhibitory concentration (MIC) of BL-P1654 as determined by the agar dilution technique and the zone of inhibition in the standard disk diffusion antibiotic susceptibility test; and (iii) the study for possible synergism between BL-P1654 and gentamicin.

MATERIALS AND METHODS

Bacteria. One hundred and seventeen clinical isolates of P. aeruginosa were tested. These included a large number of organisms known to be resistant to gentamicin and/or carbenicillin.

Antibiotic standards. Laboratory standards were obtained from Bristol Laboratories, Syracuse, N.Y. (BL-P1654 diagnostic reagents and 10-µg BL-P1654 disks); J.B. Roerig Division, Pfizer Inc., New York, N.Y. (carbenicillin diagnostic standard); and Schering Corp., Bloomfield, N.J. (gentamicin diagnostic powder).

Antibiotic susceptibility tests. The disk diffusion test was performed according to the Kirby-Bauer method (1). The MIC was determined by the agar dilution method recommended by the International Collaborative Study (ICS) on antimicrobial susceptibility testing sponsored by the World Health Organization (5). Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) was used. The inoculum was 0.002 ml of a 10−2 dilution of an overnight culture, delivered by a Steers replicator (15).

Study for possible synergism. Seventy isolates of P. aeruginosa, 24 of which were highly resistant to gentamicin (MIC > 63 µg/ml), were studied for possible synergism between BL-P1654 and gentami-
The conventional checkerboard technique (12) using the same ICS agar dilution method was employed. The antibiotics were defined as synergistic when the addition of one antibiotic in the concentration of four times less than the MIC resulted in a fourfold or more decrease in the MIC of the second antibiotic.

**RESULTS**

The correlation between the MIC of BL-P1654 versus gentamicin and BL-P1654 versus carbenicillin for the 117 clinical isolates of *P. aeruginosa* is shown in Fig. 1 and 2, respectively. Sixty-two isolates were resistant to gentamicin at 4 μg/ml, 43 were resistant to carbenicillin at 125 μg/ml, and 49 were resistant to BL-P1654 at 32 μg/ml. Fifteen of the 24 highly gentamicin-resistant (MIC > 63 μg/ml) isolates were also resistant to BL-P1654 at more than 250 μg/ml. Some gentamicin-resistant isolates were susceptible to BL-P1654 and vice versa. In general, BL-P1654 was two to eight times more active than carbenicillin against *P. aeruginosa*. However, all 28 highly carbenicillin-resistant isolates (MIC > 500 μg/ml) were also highly resistant to BL-P1654 (MIC > 250 μg/ml).

The correlation of the MIC of BL-P1654 and the zone of inhibition in the disk diffusion test is shown in Fig. 3. When an MIC of 32 μg/ml or less and a zone of inhibition of 12 mm or more were used as criteria indicating susceptibility, 10.6% of the isolates would be falsely considered resistant, whereas 4.2% of the isolates would be falsely susceptible by the disk susceptibility test.

Of the 70 clinical isolates tested for possible synergism of BL-P1654 and gentamicin, the MIC ranged from 8 to >250 μg/ml for BL-P1654 and 2 to >63 μg/ml for gentamicin. Synergy was demonstrated against 45 of the 70 isolates, but only against 4 of 24 highly gentamicin-resistant (MIC > 63 μg/ml) isolates. The combination of BL-P1654 and gentamicin acted synergistically against only 1 of 12 isolates highly resistant to BL-P1654 (MIC > 250 μg/...
ml). There was no synergism against any of the nine isolates highly resistant to both gentamicin and BL-P1654.

DISCUSSION

Human pharmacology data recently revealed that the peak serum concentration of 47 and 80.8 µg/ml were obtained after the administration of 1.0 g of BL-P1654 by a slow 1-h and rapid 15-min intravenous infusion, respectively, and that no evidence of hepatic or renal toxicity was observed in patients receiving 1 g every 4 h for 7 days (2). It seems reasonable that 32 µg/ml may be arbitrarily used to separate susceptible and resistant organisms. BL-P1654 is significantly more active than carbenicillin against P. aeruginosa in vitro, though isolates highly resistant to carbenicillin were also highly resistant to BL-P1654.

There was unsatisfactory correlation between the MIC as determined by the ICS agar dilution technique and the Kirby-Bauer disk diffusion test. Using an MIC of 32 µg/ml or less and a zone of inhibition of 12 mm or more by a 10-µg BL-P1654 disk as indicative of susceptibility, the false-resistance rate was unacceptably high. Changing the cut-off point of the zone of inhibition resulted in worse correlation. Perhaps disks containing different concentrations of this antibiotic should be tested in order to find an optimal concentration for the disk test. The same situation existed in the case of carbenicillin and three different disk concentrations (100, 50, and 25 µg) were evaluated (4, 10, 12, 14, 16, 18). The Food and Drug Administration has recently approved the 100-µg carbenicillin disk for use in the clinical laboratory.

Synergism of the combination of BL-P1654 and gentamicin was demonstrated against many but not all isolates of P. aeruginosa. Especially if an isolate was highly resistant to one of the two antibiotics, in vitro synergism was seldom demonstrated. If the isolate was highly resistant to both antibiotics, no synergism was demonstrated.

The accumulated in vitro data indicate that BL-P1654 is a promising semisynthetic penicillin which is significantly more active than carbenicillin against P. aeruginosa. Further pharmacologic and toxicologic studies are warranted.

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LITERATURE CITED