Comparative In Vitro Evaluation of BL-P1654 and Carbenicillin Against Pseudomonas

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Prior investigators have reported a broad gram-negative spectrum and a marked anti-Pseudomonas activity in vitro with BL-P1654, 6-[R-alpha-(guanylureido)phenylacetamido]-penicillanic acid. An in vitro comparison of BL-P1654 to carbenicillin was performed against 84 strains of Pseudomonas and 39 strains of Enterobacteriaceae. Mean minimal inhibitory concentrations in Mueller-Hinton broth of BL-P1654 and carbenicillin were 12.5 and 100 \( \mu g/ml \), respectively. Bactericidal studies with representative strains of Pseudomonas showed a consistent lack of bactericidal activity with BL-P1654. Mean bactericidal concentrations of BL-P1654 and carbenicillin were \( \geq 200 \) and 100 \( \mu g/ml \), respectively. The lack of bactericidal activity of BL-P1654 was further substantiated with the “killing curve” technique. Based on these data, BL-P1654 can not be considered a substitute for carbenicillin as the penicillin analogue of choice for Pseudomonas infections.

Recently, several semisynthetic penicillins have been introduced which have activity against gram-negative microorganisms (9). In particular, efforts have been made to develop substituted penicillins active against strains of Pseudomonas. Alpha-carboxybenzyl penicillin (carbenicillin) was the first penicillin analogue to achieve clinical acceptance for treatment of infections due to Pseudomonas, and its activity has been characterized elsewhere (1, 6). In addition, a number of investigators have reported parallel studies on alpha-sulfoamopenicillins (4, 7). Price et al. (7) reported that this latter group, characterized by the presence of a sulfoamino or modified sulfoamino group in the side chain, is markedly inhibitory to strains of Pseudomonas. Further, they are notably resistant against beta-lactamase destruction.

Prior investigators using BL-P1654, 6-[R-alpha-guanylureido]phenylacetamido] -penicillanic acid (5, 8, 10), have reported a marked inhibitory activity of this penicillin against strains of Pseudomonas in addition to a resistance to the beta-lactamases produced by Pseudomonas strains. However, unlike the sulfoamopenicillins, BL-P1654 retains a broad spectrum of antimicrobial activity against a large number of Enterobacteriaceae. This study was carried out to review the in vitro antimicrobial activity of this penicillin against Pseudomonas, and in particular to compare its bactericidal activity to that of carbenicillin.

**MATERIALS AND METHODS**

Collection of organisms. Eighty-four strains of Pseudomonas and 39 strains of Enterobacteriaceae were collected from patients hospitalized at Los Angeles County/University of Southern California Medical Center from January through March 1974. Representative organisms were selected only if isolated from blood, cerebrospinal fluid, urine, or body fluids, to assure the probability of involvement in pathological processes.

Susceptibility testing. The minimal inhibitory concentrations (MICs) were determined in serial dilutions of BL-P1654 (200 to 0.1 \( \mu g/ml \)) or carbenicillin (400 to 0.2 \( \mu g/ml \)) in Mueller-Hinton broth (BBL). Inocula sizes were standardized to contain \( 10^8 \) organisms per ml. The MIC was defined as the lowest concentration of antibiotic in which no visible growth occurred after 18 to 24 h of incubation at 37 C. Tubes without growth were plated on Mueller-Hinton agar (BBL) with a 3-mm loop; the minimal bactericidal concentration (MBC) was defined as the lowest concentration of antibiotic which allowed no visible growth after subsequent incubation for 24 h.

Studies of bactericidal activity. The growth of selected isolates representative of resistant and susceptible strains of Pseudomonas or Enterobacteriaceae was followed by consecutive colony counts on subcultures from Mueller-Hinton brain heart infusion (BBL), or tryptic soy (Difco) broth. Colony counts were performed at 0, 2, 4, 6, and 18 h after inoculation in broth with or without the addition of
BL-P1654 or carbenicillin. Broth was inoculated with a concentration of approximately 10^4 organisms per ml and incubated in a stationary position at 37 C. Concentrations of carbenicillin or BL-P1654 calculated to be approximately 2- to 10-fold their respective MIC's were added to the cultures.

RESULTS

Susceptibility studies. Table 1 shows the number of Pseudomonas strains susceptible to BL-P1654 at twofold increases in antibiotic concentration. Median MICs of BL-P1654 and carbenicillin were 12.5 and 100 μg/ml, respectively. Table 2 shows the MICs of BL-P1654 for 39 strains of Enterobacteriaceae. It is apparent that the susceptibility of Enterobacteriaceae to BL-P1654 was variable and not predictable for all strains.

The MICs and MBCs of BL-P1654 and carbenicillin for 14 strains of Pseudomonas are compared in Table 3. The bactericidal concentrations of BL-P1654 consistently exceeded the respective MIC's by greater than 10-fold (approximately two to three serial dilutions). In contrast, the MBC of carbenicillin was equal to or no greater than twofold its inhibitory concentration. For no strain was a lower bactericidal concentration noted for BL-P1654 compared to that determined for carbenicillin. Serial passage of strains of Pseudomonas in subinhibitory concentrations of BL-P1654 produced increases in the MIC from 6.25 to >200 mg/ml in six to seven passages. Resistance was stable and remained at high levels for 10 to 12 passages without added antibiotic.

Figure 1 compares the bactericidal activity of carbenicillin and BL-P1654 on Pseudomonas (strain 74-148) and a strain of Escherichia coli (strain 73-306) susceptible to BL-P1654 in Mueller-Hinton broth. BL-P1654 at a concentration 10-fold its MIC failed to decrease the number of colony-forming units of most strains of Pseudomonas more than a single log during 24 h of incubation despite a low MIC. In contrast, carbenicillin inhibited the growth of susceptible strains of Pseudomonas at a concentration equal to or no greater than twofold its MIC.

The lack of bactericidal activity of BL-P1654 was often observed among strains of Pseudomonas, whereas strains of Enterobacteriaceae were killed frequently with levels of BL-P1654 near the respective MIC of each strain. The effect on bactericidal activity was not specific to the media, and similar bactericidal effects were seen when brain heart infusion and tryptic soy broth were utilized.

<table>
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<tr>
<th>Table 1. Number of strains of Pseudomonas inhibited at successive concentrations of BL-P1654 and carbenicillin*</th>
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<tr>
<td>Antibiotic</td>
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<td>Carbenicillin</td>
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* Total number of strains was 84.
DISCUSSION

The isolation of the penicillin nucleus, 6-aminopenicillanic acid, by Batchelor and co-workers (2) in 1959 led to many productive attempts at changes in pharmacological and antimicrobial activity by its chemical modification. With the introduction of carbenicillin in 1967, an anti-Pseudomonas penicillin became a reality. However, the relative lack of potency of carbenicillin against Pseudomonas, which necessitates large, frequent doses and the emergence of resistant microorganisms or superinfections during therapy, has been a serious drawback.

Recently, several investigators have reviewed the antimicrobial activity of BL-P1654. Van Scoy and co-workers (10) reported that a concentration of 50 μg of BL-P1654 per ml was inhibitory for 58% of strains of Pseudomonas. Of importance, they noted a 30-fold concentration difference between inhibitory and bactericidal concentrations for strains of Pseudomonas and Proteus. However, Price and his colleagues (8) found that 75% of their strains of Pseudomonas were inhibited by 8.0 μg of BL-P1654 per ml, utilizing a nutrient agar assay system. The bactericidal activity of BL-P1654 against Pseudomonas was examined for only two strains in this latter study; however, they also noted a large disparity between MICs and MBCs. In addition, Price et al. demonstrated that resistance acquired to BL-P1654 by serial passage was cross-resistant with carbenicillin.

Bodey and co-workers (3) recently reviewed the pharmacology of BL-P1654 in humans. Mean peak serum levels of 40.7 and 80.5 μg/ml were achieved after intravenous doses of 0.5 and 1.0 g, respectively. Fifty to sixty-five percent of each dose was excreted in the urine in 6 h, and serum concentrations after a dose of 1.0 g ranged from 6 to 23 μg/ml at the end of a 4-h period. There was no evidence of accumulation of the drug over a 4-day period. However, the occurrence of renal tubular necrosis in dogs receiving large doses (100 to 400 mg/kg) was reported. High concentrations of drugs were achieved in the urine, despite a slightly slower renal excretion of BL-P1654 than most other penicillins. In addition, these investigators referred to an earlier study by Bodey and Stewart (5) in which 75% of Pseudomonas isolates were inhibited by BL-P1654 at concentrations of ≤12.5 μg/ml, and, in contrast to our findings, BL-P1654 failed to exhibit bactericidal activity in concentrations equal to its MIC in only 2% of their strains of Pseudomonas.

A nearly uniform lack of in vitro bactericidal activity against strains of Pseudomonas was noted in our study of BL-P1654. The fact that our study was carried out with recent isolates which could have acquired increased resistance may explain the divergent results. At present, these in vitro data suggest that this penicillin will have only limited use in infections due to Pseudomonas.

ACKNOWLEDGMENT

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LITERATURE CITED

