In Vitro Studies of a New Semisynthetic Penicillin,
6-(d-\(\alpha\)-Sulfoaminophenylacetamido)-
Penicillanic Acid

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The activity of 6-(d-\(\alpha\)-sulfoaminophenylacetamido)-penicillanic acid was determined against 357 clinical isolates of gram-negative bacilli by use of the tube-dilution technique. The majority of the isolates of Pseudomonas species were inhibited by 200 \(\mu\)g/ml or less of this antibiotic. Most of the isolates of Escherichia coli had a minimal inhibitory concentration of 50 \(\mu\)g/ml or less. Seventy-three percent of the isolates of P. mirabilis, 40% of the isolates of P. morganii, and 43% of the isolates of Enterobacter species were inhibited by 12.5 \(\mu\)g/ml or less, whereas most of the isolates of Klebsiella species and Serratia species were resistant. The activity of this semisynthetic penicillin was affected by the size of the inoculum. The drug was bactericidal against all isolates of E. coli and Proteus species that were sensitive to it, but it was bactericidal against only 32% of the sensitive isolates of Pseudomonas species.

The incidence of infections caused by gram-negative bacilli has increased during the last two decades (1–3). Pseudomonas species has been the causative agent in a substantial number of these infections. Patients with leukemia, metastatic cancer, cystic fibrosis, and extensive burns are particularly susceptible to Pseudomonas species infections (4, 5).

Polymyxin B sulfate and colistin sulfate have been used extensively as therapy for Pseudomonas species infections but have not been very effective in patients with impaired host defenses. Gentamicin sulfate, a new aminoglycoside antibiotic, has been very effective against Pseudomonas species infections in patients with extensive burns, but has been less effective in patients with blood dyscrasias (6). All of these drugs have potential nephrotoxicity, especially in patients with preexisting renal impairment.

Recently, penicillin derivatives have been synthesized which have antipseudomonal activity. Carbenicillin (disodium \(\alpha\)-carboxybenzylpenicillin) was the first of these semisynthetic penicillins to undergo extensive investigation (Fig. 1; 7, 8). When administered in high doses, it has been very effective against Pseudomonas species infections, even in patients with impaired host defenses (G. P. Bodey et al., in preparation). A new semisynthetic penicillin, 6-(d-\(\alpha\)-sulfoaminophenylacetamido)-penicillanic acid (BL-P1462), has been prepared by Bristol Laboratories, Syracuse, N.Y. (Fig. 1). The results of in vitro studies of this drug against clinical isolates of gram-negative bacilli are presented in this report.

MATERIALS AND METHODS

Sensitivity tests were performed on 357 clinical isolates of gram-negative bacilli by use of the tube-dilution technique (10). Organisms to be tested were incubated in Mueller-Hinton broth (BBL) at 37 C for 18 hr, and 0.1 ml of a 10\(^{-4}\) dilution of this broth was used as inoculum for isolates of Pseudomonas species. For other gram-negative bacilli, 0.1 ml of a 10\(^{-4}\) dilution was used. Serial dilutions of 6-(d-\(\alpha\)-sulfoaminophenylacetamido)-penicillanic acid were made with Mueller-Hinton broth, and the minimum inhibitory concentration (MIC) was determined after incubation at 37 C for 18 hr. All tubes containing trace growth or no discernible growth were subcultured on sheep blood-agar. The drug was considered to be bactericidal against those isolates which failed to grow on subculture of the tube containing the MIC.

All bacterial isolates used in this study were cultured from specimens obtained from patients from December 1966 to October 1968. The majority of these patients were hospitalized at this institution and had an underlying malignant disease. The sites (in the patients) from which the 160 isolates of Pseudomonas species were obtained are listed in Table 1. Seventy-one isolates of Escherichia coli, 55 of Klebsiella species,
Pseudomonas species.

The activity of 6-[(D-α-sulfoaminophenylacetamido)]-penicillic acid against isolates of Pseudomonas species was shown to be more active than carbenicillin. The results with BL-P1462 were more active than carbenicillin against 70% of the 70 isolates of Pseudomonas species, equally active against 16%, and less active against 14% (Table 2).

34 of Serratia species, 15 of P. morganii, 11 of P. mirabilis, and 11 of Enterobacter species were tested. All 197 isolates of organisms other than Pseudomonas species were obtained from cultures of blood specimens.

RESULTS

The MIC values against isolates of Pseudomonas species are shown in Fig. 2. The majority of the isolates had an MIC of 200 μg/ml or less, but only 6% had an MIC of 50 μg/ml or less. The activity of 6-[(D-α-sulfoaminophenylacetamido)]-penicillic acid (BL-P1462) and carbenicillin was determined against 70 isolates of Pseudomonas species. BL-P1462 was slightly more active than carbenicillin. The results with BL-P1462 against the 70 isolates were the same as for the total 160 isolates. BL-P1462 was more active than carbenicillin against 70% of the 70 isolates of Pseudomonas species, equally active against 16%, and less active against 14% (Table 2).
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FIG. 3. Activity of 6-(α-sulfoaminophenylacetamido)-penicillanic acid (BL-P1462) against gram-negative bacilli. The inoculum was 0.1 ml of a 10⁻³ dilution of an 18-hr broth culture.

FIG. 4. Effect of inoculum size on the activity of 6-(α-sulfoaminophenylacetamido)-penicillanic acid (BL-P1462) against Pseudomonas species.

The MIC values against isolates of other gram-negative bacteria are shown in Fig. 3. Most of the E. coli isolates had an MIC of 50 μg/ml or less. Very few isolates of Serratia species and Klebsiella species were sensitive to BL-P1462. Seventy-three per cent of the isolates of P. mirabilis, 40% of the isolates of P. morganii, and 45% of the isolates of Enterobacter species had an MIC of 12.5 μg/ml or less. Most of the remaining isolates of these organisms were resistant to 500 μg of BL-P1462 per ml.

The effect of inoculum size on MIC was determined with 20 isolates of Pseudomonas species. Dilutions, 10⁻³, 10⁻⁴, and 10⁻⁵, of an 18-hr broth culture were used as inoculum. The activity of BL-P1462 was greater with the smaller concentrations of organisms (Fig. 4). Similar results were obtained when 10 isolates of E. coli were tested (Fig. 5). BL-P1462 was inactive against 10 isolates of Klebsiella species, regardless of the inoculum size.

BL-P1462 was bactericidal against all of the sensitive isolates of Proteus species and E. coli. It was bactericidal against only 32% of the isolates of Pseudomonas species and against none of the isolates of Serratia species and Enterobacter species.

DISCUSSION

6-(α-sulfoaminophenylacetamido)-penicillanic acid (BL-P1462) is a new semisynthetic penicillin which is active in vitro against some clinical isolates of gram-negative bacilli. The
The majority of isolates of *Pseudomonas* species were inhibited by 200 μg/ml or less. This drug was slightly more active than carbenicillin against the same isolates of *Pseudomonas* species (8). However, carbenicillin was not tested simultaneously with BL-P1462, and a larger inoculum was used. The superior results obtained with BL-P1462 may have been caused by the use of a smaller concentration of organisms, since the activity of this antibiotic is dependent upon the size of the inoculum. Other investigators have observed that the size of the inoculum also affects the activity of carbenicillin, but this was not found in our study (7, 8). However, BL-P1462 was substantially more active than carbenicillin against 16% of the isolates of *Pseudomonas* species and this degree of increased activity probably cannot be explained by smaller inoculum size.

Clear end points were not always obtained when isolates of *Pseudomonas* species were tested against carbenicillin or BL-P1462. Nearly 25% of the isolates did not give a clear end point when they were first tested against BL-P1462. A well-defined MIC was obtained when the studies were repeated, and subsequent testing confirmed these results.

BL-P1462 was not as active as carbenicillin against other gram-negative bacilli. Although the drugs were not studied simultaneously, the same concentration of organisms was used for the inoculum. Over 80% of isolates of *E. coli* were sensitive to 25 μg of carbenicillin per ml, whereas only 15% were sensitive to the same concentration of BL-P1462. Carbenicillin was more active against *Proteus* species, but BL-P1462 was more active against *Enterobacter* species. *Klebsiella* species was resistant to both antibiotics.

Clinical studies have not yet been conducted with BL-P1462. Our in vitro studies suggest that it may be effective against *Pseudomonas* species infections. Carbenicillin has been very effective clinically when administered in large doses, despite its marginal activity in vitro. The clinical results with carbenicillin against *Pseudomonas* species infections have been better than in vitro tests suggest, and this may also be true for BL-P1462 (8).

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**LITERATURE CITED**