Mezlocillin: In Vitro Studies of a New Broad-Spectrum Penicillin

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Mezlocillin is a new semisynthetic penicillin that inhibited 71% of the isolates of Serratia marcescens, 67% of Escherichia coli, 50% of Enterobacter spp., and 49% of Klebsiella spp. at a concentration of 12.5 μg/ml. It is also active against both indole-positive and -negative Proteus spp. and gram-positive cocci, except penicillin G-resistant Staphylococcus aureus. At a concentration of 100 μg/ml, it inhibited 94% of the isolates of Pseudomonas aeruginosa. It is more active than ampicillin, carbenicillin, and cephalothin against some gram-negative bacilli.

Gram-negative bacilli continue to be a major cause of infection in hospitalized patients. This is especially true among patients with malignant diseases and patients receiving immunosuppressive therapy (3, 4). Often these patients do not respond, despite the in vitro susceptibility of the etiological organism to the antibiotic utilized (1). Consequently, there is a continuing need for the development of new antibiotics. The ureido-penicillins are an interesting group of semisynthetic penicillins that have broad-spectrum activity against gram-negative bacilli, including Pseudomonas aeruginosa (2). Figure 1 shows the chemical structure of mezlocillin (D-α-[2-oxo-3-mesly-imidazolidinyl]-carbonyl]-aminobenzylpenicillin; BAY 1353), a new ureido-penicillin recently undergoing investigation (H. B. König, K. G. Metzger, H. A. Offe, and W. Schrock, Prog. Abstr. Intersci. Conf., Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 372, 1974; K. Metzger, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., 332, 1975). This report presents the results of in vitro studies of mezlocillin, which indicate that it may be a potentially useful antibiotic.

MATERIALS AND METHODS

Susceptibility tests were conducted on 556 clinical isolates of gram-negative bacilli and 88 clinical isolates of gram-positive cocci, using the dilution technique with an automatic microtiter system (Canalco, Autotiter instruction manual). All organisms were inoculated into Mueller-Hinton broth (Difco) and incubated at 37°C for 18 h. For gram-negative bacilli, a 0.05-ml sample of a 10–2 dilution of this broth culture (approximately 106 colony-forming units [CFU]/ml) was used as the inoculum. For gram-positive cocci, a 0.05-ml sample of a 10–2 dilution of this broth culture (approximately 106 CFU/ml) was used as the inoculum for susceptibility testing.

All gram-negative bacilli used in this study were cultured from blood specimens obtained from patients between 1967 and 1975. The patients were hospitalized at this institution and had underlying malignant diseases. A total of 100 isolates each of P. aeruginosa, Klebsiella spp., and Escherichia coli, 98 isolates of Proteus spp., 69 isolates of Serratia spp., and 89 isolates of Enterobacter spp. were used. All gram-positive cocci used in this study were cultured from specimens obtained from hospitalized patients, most of whom did not have cancer. A total of 22 isolates of Staphylococcus pyogenes, 14 isolates of S. pneumoniae, and 62 isolates of Staphylococcus aureus were used. The susceptibility of isolates of S. aureus to penicillin G was determined by the broth dilution method. Isolates inhibited by less than 0.10 μg/ml were selected as penicillin G susceptible, and those isolates resistant to more than 25 μg/ml were selected as penicillin G resistant.

Mezlocillin (BAY 1353) was supplied as a powder by Delbay Pharmaceuticals, Inc., Bloomfield, N.J. Ampicillin and BL-P1654, 6-[D-α-[3-guanylureido]-phenylacetamido] penicillin acid, were supplied by Bristol Laboratories, Syracuse, N.Y. Carbenicillin and ticarcillin were supplied by Beecham Pharmaceuticals, Bristol, Tenn. Twofold serial dilutions of the antibiotics were made with Mueller-Hinton broth, and the minimal inhibitory concentration (MIC) was determined after incubation at 37°C for 18 h. All wells containing trace growth or no discernible growth were subcultured on sheep blood agar. A calibrated pipette was used to transfer 0.01 ml of the inoculum. The minimum bactericidal concentration (MBC) was determined after incubation at 37°C for 18 h. The MBC was defined as the lowest concentration of drug that yielded less than 50 colonies on subculture (less than 5 colonies/0.001 ml of inoculum). Comparison studies were conducted simultaneously.
RESULTS

The in vitro activity of mezlocillin against gram-positive cocci and gram-negative bacilli is shown in Fig. 2. Although the majority of isolates of S. pneumoniae were quite susceptible to mezlocillin, 14% required concentrations of 6.25 to 25 μg/ml to inhibit their growth. Likewise, the majority of penicillin G-susceptible S. aureus were inhibited by 0.2 μg or less of mezlocillin per ml, whereas penicillin G-resistant S. aureus were also resistant to mezlocillin. Isolates of S. pyogenes were quite susceptible to mezlocillin. Over 95% of the isolates of P. mirabilis and 70% of the isolates of indole-positive Proteus spp. were susceptible to 1.56 μg or less of mezlocillin per ml. A concentration of 12.5 μg/ml inhibited 71% of the isolates of S. marcescens, 67% of E. coli, 50% of Enterobacter spp., and 49% of Klebsiella spp., but only 27% of P. aeruginosa. However, at a concentration of 100 μg/ml, mezlocillin inhibited 94% of the isolates of P. aeruginosa. Generally, the MIC was also the MBC, but this was not true for P. aeruginosa. The MBC for most isolates of P. aeruginosa was twice as high as the MIC.

The effect of inoculum size on the MIC and MBC was determined for 10 isolates each of K. pneumoniae, E. coli, and P. aeruginosa (Fig. 3). For all isolates of K. pneumoniae and most
isolates of E. coli, the MIC and MBC were the same. Inoculum size had the greatest effect on the activity of mezlocillin against K. pneumoniae. Using an inoculum of 10⁵ CFU/ml, all isolates were inhibited at a concentration of 6.25 µg/ml, whereas using an inoculum of 10⁷ CFU/ml, none was inhibited at a concentration of 400 µg/ml. The MIC and MBC for isolates of E. coli increased sixfold or more when the inoculum size was increased from 10⁵ to 10⁷ CFU/ml. All of the isolates of P. aeruginosa were inhibited by 100 µg or less of mezlocillin per ml when the inoculum was 10⁵ CFU/ml, but only 30% were inhibited by 400 µg/ml when the inoculum was 10⁷ CFU/ml.

The effect of pH on the susceptibility of 10 isolates each of E. coli, K. pneumoniae, and P. aeruginosa is shown in Fig. 4. The pH was adjusted by the addition of phosphate buffer to Mueller-Hinton broth. The greatest effect was observed against isolates of E. coli, which were more susceptible at alkaline pH. The pH had no appreciable effect on the activity of mezlocillin against isolates of P. aeruginosa. Media had little effect on the activity of mezlocillin (Fig. 5). However, isolates of K. pneumoniae, E. coli,
and *P. aeruginosa* were slightly more resistant when tested in nutrient broth, with the exception of one isolate of *E. coli* which was considerably more susceptible to mezlocillin when tested in nutrient broth.

The activity of mezlocillin against 50 isolates each of various gram-negative bacilli was compared with ampicillin, carbenicillin, and cephalothin (Fig. 6 to 10). Figure 10 shows similar data for 21 isolates of indole-positive *Proteus* spp. Mezlocillin was the most active drug against isolates of *E. coli*, although about 30% of the isolates were resistant to 25 μg/ml and some of these isolates were more susceptible to cephalothin. Cephalothin was the most active antibiotic against *K. pneumoniae*, although mezlocillin was the most active penicillin. Mezlocillin was substantially more active than any of the other antibiotics against *S. marcescens*. Cephalothin was virtually inactive, whereas carbenicillin was active against nearly 50% of these isolates. Mezlocillin was the most active antibiotic against *Enterobacter* spp. although 56% of the isolates were resistant to 25 μg/ml. Carbenicillin was the second most active antibiotic, but 74% of the isolates were resistant to 25 μg/ml of this drug per ml. All three penicillins were equally active against *P. mirabilis*. Two isolates that were resistant to ampicillin and cephalothin and one isolate that was relatively resistant to carbenicillin were inhibited by 6.25 μg of mezlocillin per ml. Mezlocillin was also the most active antibiotic against indole-positive *Proteus* spp. and was consistently four times more active than carbenicillin. Ampicillin and cephalothin had minimal activity against these isolates.

The activity of mezlocillin was compared with that of BL-P1654, carbenicillin, and ticarcillin against 50 isolates of *P. aeruginosa* (Fig. 11). The most active antibiotic was BL-P1654 and the least active was carbenicillin. Mezlocillin and ticarcillin had comparable activity.

**DISCUSSION**

Mezlocillin is a broad-spectrum semisynthetic penicillin with activity against both gram-positive cocci and gram-negative bacilli. It is of particular interest because it is marginally active against *S. marcescens, K. pneumoniae,* and *P. aeruginosa*. It is more active in vitro than ampicillin or carbenicillin against *E. coli* and as active as these two penicillins against *P. mirabilis*. It is also more active in vitro than carbenicillin against indole-positive *Proteus* spp. and *Enterobacter* spp. It is as active as cephalothin against 80% of the isolates.

![Comparative Activity of Antibiotics Against Escherichia coli](image1.png)

**Fig. 6.** Comparative activity of antibiotics against 50 isolates of *E. coli*. An inoculum of 10⁵ CFU/ml was used.

![Comparative Activity of Antibiotics Against Klebsiella spp](image2.png)

**Fig. 7.** Comparative activity of antibiotics against *K. pneumoniae*. An inoculum of 10⁵ CFU/ml was used.
**Fig. 8.** Comparative activity of antibiotics against *S. marcescens*. An inoculum of $10^5$ CFU/ml was used.

**Fig. 9.** Comparative activity of antibiotics against Enterobacter spp. An inoculum of $10^5$ CFU/ml was used.

**Fig. 10.** Comparative activity of antibiotics against Proteus spp. Fifty isolates of *P. mirabilis* (a) and 21 isolates of indole-positive Proteus spp (b) were tested. An inoculum of $10^5$ CFU/ml was used.
of Klebsiella spp. and is the most active of the four antibiotics against S. marcescens. However, another ureido-penicillin, BP-P1654, is more active than mezlocillin in vitro against P. aeruginosa. The ureido-penicillins have been shown to be relatively resistant to destruction by the β-lactamase of isolates of E. coli and S. marcescens resistant to ampicillin, which may account for the better in vitro results with mezlocillin (B. Wiedemann, V. Kremery, and H. Knothe, Int. Kongr. Chemother., 9th, London, Abstr. M366, 1975).

There has been controversy in the past over the bactericidal activity of ureido-penicillins. Generally, mezlocillin was bactericidal at the MIC, but this was not true for isolates of P. aeruginosa. Inoculum size had a major effect on the activity of mezlocillin. It was ineffective against all isolates of K. pneumoniae and most isolates of P. aeruginosa when a large inoculum was used. This suggests that these cells are not uniformly susceptible to mezlocillin, and a large inoculum includes more cells that are inherently resistant to the antibiotic. This adverse effect of inoculum size has been observed with other penicillins (5, 6).

Mezlocillin is an interesting new penicillin because of its broad-spectrum activity. The drug deserves further evaluation because it is potentially more active than ampicillin, carbenicillin, and cephalothin against some gram-negative bacilli.

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