In Vitro Activity of Carbenicillin Against Gram-negative Bacilli

GERALD P. BODEY and LOIS M. TERRELL
The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025

Received for publication 13 February 1968

The activity of a new semisynthetic penicillin, carbenicillin, was determined against 241 strains of gram-negative bacilli with the tube-dilution technique. Of 143 strains of Pseudomonas sp., 99 had a minimal inhibitory concentration of 200 to 300 \( \mu \text{g/ml} \). The majority of strains of Escherichia coli and Proteus spp. were sensitive to this antibiotic, with minimal inhibitory concentrations of 25 \( \mu \text{g/ml} \) or less. Strains of Klebsiella sp. were quite resistant to carbenicillin. The size of inoculum had no significant effect on the minimal inhibitory concentration for Pseudomonas sp.

Infections caused by Pseudomonas sp. have been increasingly recognized in recent years. These infections have occurred most frequently in patients with extensive burns, in patients undergoing urological procedures, and in patients with hematological malignancies. Pseudomonas sp. is responsible for more than 30% of the episodes of fatal septicemia in patients with acute leukemia (4).

Polymyxin B sulfate and colistin sulfate have not been very effective in the treatment of Pseudomonas sp. infections in patients with altered host defenses (G. P. Bodey unpublished data). Gentamicin sulfate has been effective in the treatment of these infections in patients with extensive burns (5) but has been less effective in patients with leukemia. Hence, new antibiotics with anti-pseudomonal activity are needed.

Carbenicillin (disodium \( \alpha \)-carboxybenzylpenicillin) is a new semisynthetic penicillin with a wide spectrum of activity against gram-positive and gram-negative organisms (1). Studies of carbenicillin conducted in Great Britain have demonstrated marginal activity against Pseudomonas sp. This paper reports the results of in vitro studies on the activity of carbenicillin against strains of Pseudomonas sp. and other gram-negative bacilli.

MATERIALS AND METHODS

Sensitivity testing was assessed on 241 strains of gram-negative bacilli with the tube-dilution technique (3). Organisms to be tested were incubated in Mueller-Hinton broth at 37°C for 18 hr, and 0.1 ml of \( 10^{-8} \) dilution of this broth was used as inoculum. Fifty-one strains of Pseudomonas sp. were also tested with inoculum of 0.1 ml of \( 10^{-4} \) dilution of an 18-hr culture. Serial dilutions of carbenicillin (Beecham Pharmaceuticals, Clifton, N.J.) were made with Mueller-Hinton broth (BBL), and the minimal 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. Carbenicillin was considered bactericidal for those strains that failed to grow on subculture of the tube containing the minimal inhibitory concentration.

All strains of organisms used in this study were obtained from cultures of patient specimens. The majority of these patients were hospitalized at this institution and had an underlying malignant disease. Table 1 lists the patient sites from which the 143 strains of Pseudomonas sp. were obtained. In addition, the following were tested: 34 strains of Escherichia coli, 24 strains of Klebsiella sp., 17 strains of Proteus spp., 16 strains of Serratia spp., and 7 strains of Enterobacter spp. All of these specimens were obtained from blood cultures.

RESULTS

The MIC for all strains of Pseudomonas sp. are listed in Table 2. Only 4% of these organisms had a MIC of 50 \( \mu \text{g/ml} \) or less. The MIC for the majority of strains was between 200 and 300 \( \mu \text{g/ml} \). Carbenicillin was bactericidal for only 39 strains.

To determine the effect of inoculum size on MIC, 51 strains of Pseudomonas sp. isolated from blood cultures were also tested with a smaller number of organisms in the inoculum. The results, using 0.1 ml of a \( 10^{-6} \) dilution of an 18-hr broth culture, are listed in Table 3. The MIC values were similar to those found with the larger inoculum—the majority of strains had an MIC of 200 to 300 \( \mu \text{g/ml} \). Comparing the two sizes of...
inoculum, the MIC for 39 strains was either identical or varied by only one tube dilution. The MIC was higher as often as it was lower when the smaller inoculum was used.

The effect of duration of incubation on the activity of carbenicillin against *Pseudomonas* sp. was studied. Ten strains of *Pseudomonas* sp. with an MIC of 300 μg/ml were incubated with 200 μg of carbenicillin per ml and subcultured every 2 hr for 18 hr (Table 4). For the majority of strains, the number of viable organisms remained constant during the first 6 hr after inoculation. Maximal inhibition by carbenicillin was usually observed at 10 to 12 hr after inoculation, and there was a median reduction of 2 logs in the number of viable organisms (range 1 to 6 logs). Between 14 and 18 hr there was an increase in the number of viable organisms present in eight of the ten strains. This increase varied from 1 to 5 logs.

The MIC for other gram-negative bacilli are listed in Table 5. The majority of strains of *E. coli* had an MIC of 12.5 to 25 μg/ml. *Proteus morganii* was somewhat more resistant than *P. mirabilis*, but the majority of strains had MIC values of 12.5 μg/ml or less. The MIC values for strains of *Serratia* spp. were variable, but 63% were 100 μg/ml or greater. *Klebsiella* sp. were uniformly resistant to carbenicillin; 92% of the strains had MIC values greater than 500 μg/ml. Only a few strains of *Enterobacter* spp. were available for testing, but they were more sensitive than *Klebsiella* sp. Carbenicillin was bactericidal for 35 of the 60 strains with MIC values of 100 μg/ml or less. Similar results were found for all the species of organisms.

**DISCUSSION**

The results of this study are not as encouraging as those reported from Great Britain. Acred et
Table 5. Activity of carbenicillin against gram-negative bacilli

<table>
<thead>
<tr>
<th>Minimal inhibitory concn (µg/ml)</th>
<th>No. of strains inhibited*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.56</td>
<td>—</td>
</tr>
<tr>
<td>3.12</td>
<td>—</td>
</tr>
<tr>
<td>6.25</td>
<td>1</td>
</tr>
<tr>
<td>12.5</td>
<td>14</td>
</tr>
<tr>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>200</td>
<td>—</td>
</tr>
<tr>
<td>300</td>
<td>—</td>
</tr>
<tr>
<td>400</td>
<td>—</td>
</tr>
<tr>
<td>500</td>
<td>—</td>
</tr>
<tr>
<td>&gt;500</td>
<td>4</td>
</tr>
</tbody>
</table>

* Number in parenthesis indicates total number of strains tested.

al. (1) studied 74 strains of Pseudomonas pyocyanea and found that 68 strains had an MIC of 50 µg/ml or less. Brumfitt et al. (2) determined the MIC of 99 strains of Pseudomonas sp.; 81% had an MIC of 100 µg/ml or less. In our study only 8% of 143 strains of Pseudomonas sp. had an MIC of 100 µg/ml or less.

The explanation for these divergent results is not readily apparent. Although Acred et al. (1) used the agar plate technique, Brumfitt et al. (2) used the tube-dilution method which was used in this study. It is possible that different types of media might affect the activity of carbenicillin. The majority of strains of Pseudomonas sp. used in our study were obtained from patients with underlying malignancies, and many of these strains caused fatal infections. These organisms may be quite virulent and inherently more resistant to antibiotics, such as carbenicillin.

Acred et al. (1), by use of the agar plate technique, found that the MIC for carbenicillin depended upon the size of inoculum used. This was not true in our study, for there was no consistent difference in MIC when 0.1 ml of 10⁻³ dilution of an 18-hr culture was used compared to 0.1 ml of a 10⁻³ dilution of an 18-hr culture.

There were some difficulties in testing the activity of carbenicillin against strains of Pseudomonas sp. with the tube-dilution technique. Some of our strains, on repeated testing, did not have a consistently sharp end point, but trace growth was observed at concentrations of carbenicillin several tubes above the previously determined MIC. This persistence of trace growth was apparent for four strains with the smaller inoculum and for nine strains with the larger inoculum. Occasionally, tubes appeared to have trace growth even when no viable organisms were recovered on direct subculture or subculture after diluting in broth. Sharp end points were always observed with the other gram-negative bacilli tested.

It has been demonstrated that, although 50 µg of carbenicillin per ml killed 99% of the original inoculum at 7 hr, resumption of growth occurred thereafter (1). The surviving organisms had not developed increased resistance to carbenicillin. In our study, when organisms were incubated with carbenicillin at a concentration below the MIC, growth was inhibited at 10 to 12 hr, with a 10⁻¹ to 1,000,000-fold reduction in the number of viable organisms. Six hr later, 80% of the strains demonstrated loss of inhibition with a 10⁻¹ to 100,000-fold increase in the number of viable organisms. Hence, carbenicillin inhibited growth of Pseudomonas sp. after 10 to 12 hr of incubation at a concentration below the MIC.

Our results with other gram-negative bacilli were similar to those of Brumfitt et al. (2). They found that 89% of Proteus spp. and 44% of E. coli had an MIC below 12.5 µg/ml, but Klebsiella sp. was uniformly quite resistant. Acred et al. (1), by use of the agar plate technique, found E. coli and Proteus spp. to be more sensitive.

A few clinical studies have been conducted with carbenicillin. Brumfitt et al. (2) treated 74 patients with gram-negative bacilli infections. Most patients had urinary-tract infections. Good results were obtained with carbenicillin against infection caused by Proteus spp. and E. coli. Only 18 of 45 patients with Pseudomonas sp. infections were cured and only 2 of 7 with Pseudomonas sp. septicemia.

Although the majority of our strains of Pseudomonas sp. were not very sensitive to carbenicillin in vitro, it is possible that this agent will be of value in the treatment of Pseudomonas sp. infections. The drug is nontoxic, and large doses may be given intravenously. Blood levels of 100 µg/ml were obtained in humans 0.5 hr after the rapid intravenous infusion of 1 g of carbenicillin (1). Since carbenicillin is largely excreted in the urine, higher blood levels may be obtained with probenecid. Clinical studies are planned to determine whether this agent, alone or in combination with other anti-pseudomonal antibiotics, will eradicate Pseudomonas sp. infections.

Acknowledgment

This investigation was supported by Public Health Service grant 10042-02 from the National Cancer Institute.
LI TERATURE CITED


