BRL-36650: In Vitro Studies and Assessment of Serum Bactericidal Activity after Single-Dose Administration in Volunteers

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Ten healthy volunteers received 1 g of BRL-36650, a new formamido-penicillin derivative, given by intravenous infusion over 15 min. Levels in serum were measured microbiologically, 30, 60, and 120 min after the start of the 15-min infusion and were (mean ± standard deviation) 102.7 ± 20.4, 59.7 ± 11.5, and 9.6 ± 1.9 mg/liter, respectively. A total of 10 strains each of Escherichia coli, Klebsiella pneumoniae, Serratia spp., and Enterobacter spp. and 14 strains of Pseudomonas aeruginosa were selected according to their susceptibility or resistance to piperacillin for the study of serum bactericidal activity (SBA). The MICs and MBCs of these strains were influenced by the choice of medium. Median SBA against E. coli and K. pneumoniae were ≥1:2,048 and 1:512, respectively. The SBA against piperacillin-susceptible Serratia spp. (1:256), Enterobacter spp. (1:128), and P. aeruginosa (1:32) were significantly higher than against piperacillin-resistant strains (1:32, 1:5, and 1:4, respectively). Killing curves confirmed the high bactericidal activities obtained against the majority of strains. In the case of one Enterobacter sp. and one P. aeruginosa isolate with an MBC ≥32, the absence of killing was noted.

Severe gram-negative bacillary infection remains a major problem in patients with cancer, especially in neutropenic patients (11).

The recent introduction of a number of new broad-spectrum penicillins and cephalosporins has generated increased interest in the management of these infections (5, 8). BRL-36650, a 6-α-formamido-penicillin derivative, has been reported to be highly active against aerobic gram-negative bacteria and stable to β-lactamases produced by these organisms (2).

In the present study, we studied the in vitro activity of BRL-36650 against clinical isolates of gram-negative bacteria. We also evaluated the efficacy, as measured by the titration of the serum bactericidal activity (SBA), and the rate of bactericidal activity in serum of parenteral BRL-36650 administered to volunteers against selected gram-negative pathogens.

MATERIALS AND METHODS

Antibiotics. BRL-36650 was kindly provided by Beecham Pharmaceuticals, European Division, and piperacillin was from Lederle-Cyanamid.

Organisms. A total of 10 strains each of Escherichia coli (7 susceptible to piperacillin, MIC, 64 mg/liter; 3 resistant to piperacillin, MIC, >64 mg/liter), Klebsiella pneumoniae (7 susceptible and 3 resistant to piperacillin), Enterobacter spp. (5 susceptible and 5 resistant to piperacillin), Serratia spp. (5 susceptible and 5 resistant to piperacillin), and 14 strains of Pseudomonas aeruginosa (5 susceptible and 2 resistant to piperacillin) were studied. The majority of them were isolated from clinical specimens from patients hospitalized in the Institut Jules Bordet. Three multiresistant (resistant to piperacillin, ceftazidime, and gentamicin and susceptible to imipenem and amikacin) clinical isolates of P. aeruginosa and five multiresistant isolates of Enterobacter spp. were provided by Beecham Pharmaceuticals, European Division.

Susceptibility tests in agar. MICs were determined by serial dilution in Mueller-Hinton 2 (MH) agar (Becton Dickinson and Co., Paramus, N.J.). A final inoculum of 10^4 CFU per spot was applied to the agar surface with a multipoint inoculator (Dynatech Laboratories, Inc., Alexandria, Va.). Plates were incubated at 37°C for 18 h. The MIC was defined as the lowest concentration of antibiotic that inhibited growth of bacteria (1).

Susceptibility tests in liquid medium. The MICs and MBCs of BRL-36650 were determined by microtitre serial dilution in MH broth (Becton Dickinson) and in a mixture of 50% broth-pooled normal human serum. Each test was done in duplicate. The final inoculum in each well was adjusted turbidimetrically to obtain 10^8 CFU/ml. The actual inoculum was controlled in 15 experiments, and the mean was 2.8 × 10^6 CFU/ml, with a range of 2 × 10^5 to 6 × 10^7 CFU/ml. MBC determination was made by subculturing 4 μl from each well on drug-free agar. The criteria for MBC were a 99.9% reduction of the original inoculum (6), taking into account the actual mean inoculum, a double sampling of 4 μl, and a total error on the volume and the initial inoculum estimation of 25%. This corresponded to 99.9% killing, with a sensitivity of 99.9% and a specificity of 99.7% (12).

Volunteers. Ten healthy volunteers (five women, five men; mean height, 1.75 m; range, 1.60 to 1.89 m; mean weight, 72 kg, range, 54 to 92 kg) were entered into the study after informed consent was obtained. Prior to the study the following blood tests were done: leukocyte, erythrocyte, hematocrit, hemoglobin, differential formula, ionogram, aspartate- and alanine-amino transferases, alkaline phosphatases, lactate dehydrogenase, total bilirubin, urea, creatinine, and uric acid. These tests were not performed after the study in the absence of clinically apparent side effects.

Administration of antibiotic. Each volunteer received 1 g of BRL-36650, diluted in saline, given by a short (15-min) intravenous infusion. Blood samples were taken at 30 min, 1 h, and 4 h after the start of infusion. The serum was separated from clotted blood and immediately stored at
SBA. SBA against all the selected strains was measured for each serum sample taken at 30 min after the start of infusion. Serum titration was done by using a mixture of broth-normal human serum as the diluent (7). Inoculum concentration and sampling for bactericidal determination were the same as described above.

All strains were tested for serum sensitivity by using a control serum obtained from each volunteer prior to the administration of antibiotics. None of the strains that were selected was inhibited.

**Rate of killing in serum.** Two strains each of *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *Enterobacter* spp. and *Serratia* sp. (one susceptible, one resistant to piperacillin) were used. Fractions of each serum sample obtained at 30 min were pooled and diluted 1:2 (4) in MH broth to a fixed volume of 2 ml. All tubes were agitated throughout the experiment on a rotator at 37°C. After 0, 2, 4, 6, and 24 h of incubation, 10 μl was removed with a calibrated loop, adequately diluted, and subcultured on MH agar. Colonies were counted after an overnight incubation at 37°C.

**RESULTS**

In Table 1 is compared the in vitro susceptibility of the strains to BRL-36650 in different media. MICs in broth of *E. coli, K. pneumoniae*, and *Enterobacter* spp. were significantly higher than those obtained in agar (P ≤ 0.01, Wilcoxon matched pairs rank test).

The addition of 50% human serum to the broth reduced the median MICs for all species. MBCs in broth were similar to the corresponding MICs, however in 50% broth-serum, significant discrepancies were observed in a few strains, showing the following very high MBC/MIC ratios: one *K. pneumoniae* isolate (MBC/MIC = 20), two *Serratia* spp. (200 and 800), and four *P. aeruginosa* isolates (16, 32, 64). Nevertheless, except for two strains of *P. aeruginosa*, MBCs for these two strains were ≤16 mg/liter. These discrepancies were confirmed by testing the strains by a macromethod in acid-washed borosilicate glass tubes (9).

Median MBCs of piperacillin-resistant strains of *K. pneumoniae, Enterobacter* spp., *Serratia* spp., and *P. aeruginosa* were higher than those of piperacillin-susceptible strains either in broth or in 50% broth-serum.

Clinical adverse effects were not observed after intravenous administration of BRL-36650 to volunteers. The serum levels are shown in Table 2.

The serum bacteriostatic activity and SBA are listed in Table 3. High bacteriostatic activities were observed against all species tested. In the case of *E. coli* and *K. pneumoniae*, median SBAs were ≥1:2,048 and 1:512, respectively. Median SBAs against *Enterobacter* spp., *Serratia* spp., and *P. aeruginosa* susceptible to piperacillin were excellent, with 94 to 100% of sera providing SBAs ≥1:8. When sera were tested against piperacillin-resistant *Enterobacter* spp. and *Serratia* spp., SBAs were lower than those of susceptible strains (1:8 and 1:64, respectively). Against piperacillin-resistant *P. aeruginosa*, the median SBA was 1:4, with only 43% of sera providing a SBA ≥1:8.

In Fig. 1 is summarized the data on the killing rates. The high rate of killing observed against a strain of *Serratia* spp. (Fig. 1A) is representative for all the tested organisms, except for one strain of *Enterobacter* spp. (Fig. 1B) and one strain of *P. aeruginosa* (Fig. 1C), both of which were resistant to piperacillin, for which the MBCs in the presence of 50% human serum were ≥32 mg/liter.

**DISCUSSION**

All strains tested were found to be susceptible to BRL-36650 in vitro. Peak serum concentrations were 7- to 100-fold higher than the MICs obtained in agar. The MICs in 50% human serum were lower than the MICs in broth, and the MBCs were very close to the MICs in both media, with the exception of a few strains for which the MIC/MBC ratios in 50% serum-broth were very high.

The SBA has been shown to predict the outcome of bacteremic infections in cancer patients (10). The sera of volunteers receiving 1 g of BRL-36650 provided good bactericidal activities against the majority of strains tested. Nevertheless, the SBA was significantly lower for piperacillin-resistant strains of *Serratia* spp., *Enterobacter* spp., and *P. aeruginosa* than for piperacillin-susceptible strains. The increased MBCs of piperacillin-resistant strains, particularly in the case of *P. aeruginosa* in the presence of human serum, could explain these differences. Although the low protein binding of BRL-36650, which is 30% (Beecham Pharmaceuticals Research Division, unpublished data), cannot explain the difference in vitro susceptibility between serum and serum-broth. Taking into account the serum levels, the bactericidal activity would probably be augmented with higher dosages of BRL-36650. Doses of up to 4.0 g were given to healthy volunteers without any trouble. Peak serum

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### Table 1. Activity of BRL-36650 against the strains selected for the SBA study, in agar and liquid media

<table>
<thead>
<tr>
<th>Species (no. of strains)</th>
<th>Серия (номера)</th>
<th>Median activity (range [mg/liter]) in:</th>
<th>Серия (номера)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agar, MIC</td>
<td>Broth</td>
<td>Broth-serum (1:1)</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td><em>E. coli</em> (10)</td>
<td>0.05 (0.02-0.05)</td>
<td>0.5 (0.02-1)</td>
<td>≤0.02 (≤0.02-0.2)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (10)</td>
<td>0.05 (0.02-4)</td>
<td>2 (0.02-16)</td>
<td>0.02 (0.02-0.05)</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp. (10)</td>
<td>2 (2-32)</td>
<td>4 (0.5-32)</td>
<td>1 (≤0.02-16)</td>
</tr>
<tr>
<td><em>Serratia</em> spp. (10)</td>
<td>1 (0.02-8)</td>
<td>0.5 (0.05-16)</td>
<td>0.02 (0.02-4)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (14)</td>
<td>2 (1-16)</td>
<td>2 (1-16)</td>
<td>1 (0.2-8)</td>
</tr>
</tbody>
</table>

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### Table 2. Serum concentration of BRL-36650 in 10 volunteers receiving a 1-g dose

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mean serum concn (mg/liter) ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>after start of infusion</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>102.7 ± 28.4 (76-108)</td>
</tr>
<tr>
<td>60</td>
<td>59.7 ± 11.5 (42-76)</td>
</tr>
<tr>
<td>240</td>
<td>9.6 ± 1.9 (8-14)</td>
</tr>
</tbody>
</table>
concentrations were found to be proportional to the given dose being respectively 57.8, 110.6, 224.7 and 427.0 mg/liter after 0.5, 1.0, 2.0, and 4.0 g, respectively, was administered as a 3-min intravenous bolus injection in 10 volunteers (Beecham Pharmaceuticals Research Division, unpublished data).

In conclusion, the high in vitro activity and its effective bactericidal activity after administration to volunteers make BRL-36650 a candidate for the treatment of gram-negative bacillary infections, and clinical trials are warranted.

LITERATURE CITED