Susceptibility of *Haemophilus influenzae* Type b to Cefatrizine, Ampicillin, and Chloramphenicol

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The susceptibility of 269 isolates of *Haemophilus influenzae* type b to cefatrizine (BL-S640), ampicillin, and chloramphenicol was evaluated by disk diffusion susceptibility tests, using a modified Bauer-Kirby method. Broth dilution susceptibility tests were performed on 88 of these isolates, including all isolates resistant by disk to cefatrizine or ampicillin. Of the six isolates resistant by disk to cefatrizine (zone size, <16 mm), four were resistant to ampicillin (zone size, <19 mm), and none were resistant to chloramphenicol (zone size, <17 mm). Only two of the six isolates of *H. influenzae* that were resistant to cefatrizine by disk were resistant to more than 4 µg of drug per ml. The four organisms resistant to ampicillin on disk were resistant, when tested by the broth method, to >128 µg/ml. These four ampicillin-resistant *H. influenzae* were susceptible to <4 µg of cefatrizine per ml. The two isolates resistant to >4 µg of cefatrizine per ml were susceptible to 0.5 and 2 µg of ampicillin, respectively, per ml. The activity of cefatrizine appears to be comparable in vitro to ampicillin against *H. influenzae*.

Cefatrizine (BL-S640) is a new semisynthetic cephalosporin compound which is water soluble and well absorbed by animals after oral administration (4). In vitro, cefatrizine appears to be four- to eightfold more active than cephalaxin against most gram-positive bacteria, as well as against *Escherichia coli* and *Klebsiella, Salmonella*, and *Shigella* sp., and approximately 16-fold more active against *Proteus mirabilis* and *Proteus vulgaris* strains (1, 3, 5, 7). It is also inhibitory in concentrations theoretically achievable in humans against many strains of cephalaxin-resistant *Enterobacter* sp. and *P. vulgaris* (6) but is inactive against *Pseudomonas aeruginosa, Serratia marcescens*, and methicillin-resistant staphylococci (1, 3, 5-7).

Efficacy trials of oral cefatrizine have been initiated in adults and, most recently, in children with cervical lymphadenitis and otitis media. Thus, specific information regarding the susceptibility of *Haemophilus influenzae* to cefatrizine is required. In previous studies in which the susceptibility of *H. influenzae* to cefatrizine has been evaluated, results have been somewhat contradictory (1, 3, 5, 7). A relatively small number of isolates have been tested, however.

**MATERIALS AND METHODS**

Between July 1974 and January 1976 *H. influenzae* type b were recovered on 269 occasions from the blood (55), cerebrospinal fluid (21), fluid obtained by needle tympanocentesis (17), or the throat (176) of children at St. Louis Children's Hospital. These isolates came from 263 patients (in six cases, positive blood and cerebrospinal fluid isolates were obtained from the same individual). These isolates were identified on the basis of their requirements for both hemin (x factor) and nicotinamide adenine dinucleotide (v factor), indole production, and lack of hemolysis on tryptic soy agar with 5% rabbit blood. The identity was confirmed by agglutination with type-specific antisera (Hyland Laboratories).

Disk diffusion susceptibility tests were performed on all isolates, using a modified Bauer-Kirby technique (2) against cefatrizine (30 µg), ampicillin (10 µg), and chloramphenicol (30 µg). Mueller-Hinton agar supplemented with 1% IsoVitalex and 1% hemoglobin was streaked with a broth culture (Mueller-Hinton broth with 10% Fildes extract) that had been adjusted to equal 0.5 McFarland standard. The appropriate antimicrobial disks were then dropped onto the inoculated plates. The plates were incubated in 5% CO₂ at 35°C for 18 h. Zone sizes were measured to determine the susceptibility of the organism to the antimicrobial agent.

Broth dilution susceptibility tests were performed on 88 of these isolates, using Mueller-Hinton broth with 10% Fildes extract. The inoculum was adjusted to approximately 10⁵ organisms/ml. Results were determined after a period of 18 h in an aerobic incubator. Cefatrizine-resistant organisms were defined as those for which the minimal inhibitory concentration (MIC) of cefatrizine was greater than 4 µg/ml, ampicillin-resistant organisms were defined...
as those for which the MIC of ampicillin was greater than 2 μg/ml, and chloramphenicol-resistant organisms were defined as those for which the MIC of chloramphenicol was greater than 8 μg/ml.

Cefatrizine is presently being evaluated by us at St. Louis Children's Hospital in children with cervical lymphadenitis. A daily dose of 40 mg/kg per day is administered in four divided oral doses. Concentrations of cefatrizine in serum are measured at 30 min and at 1, 2, 4, and 6 h after the initial dose of 10 mg/kg.

RESULTS

Six of 269 isolates of H. influenzae were resistant by disk to cefatrizine (zone size, <16 mm). Four of these isolates were resistant to ampicillin (zone size, <19 mm). None of these isolates was resistant to chloramphenicol (zone size, <17 mm).

Results of broth dilution susceptibility tests (88 isolates) against cefatrizine, ampicillin, and chloramphenicol, including all isolates that proved to be resistant by disk to cefatrizine or ampicillin, are shown in Table 1. Eighty-five of the 88 H. influenzae tested were inhibited and killed by 4.0 μg of cefatrizine per ml. The MIC and minimal bactericidal concentration of cefatrizine was 16 μg/ml for one isolate; the MICs and minimal bactericidal concentrations of cefatrizine were 32 and 64 μg/ml and 4 and 8 μg/ml, respectively, for two other resistant isolates. Eighty-four of the isolates were inhibited and killed by 2.0 μg or less of ampicillin per ml. The MIC and minimal bactericidal concentration of ampicillin was >128 μg/ml for the four resistant H. influenzae isolates. None of the strains of H. influenzae tested was resistant to concentrations of chloramphenicol that generally can be achieved in vivo. The susceptibility of H. influenzae to cefatrizine, as measured by the Bauer-Kirby technique (2) and by the broth dilution procedure, is shown in Table 2.

Six H. influenzae were resistant to cefatrizine by disk diffusion susceptibility tests; only two of these isolates were resistant to more than 4.0 μg of cefatrizine per ml. The four organisms that appeared to be resistant to ampicillin by disk proved to be resistant by broth dilution tests at >128 μg of ampicillin per ml. These findings were confirmed by the Center for Disease Control, Atlanta, Ga. The four isolates of H. influenzae resistant to ampicillin were susceptible to cefatrizine at <4 μg/ml. The two isolates resistant to cefatrizine at 16 and 32 μg/ml, respectively, were susceptible to ampicillin in concentrations of 0.5 and 2 μg/ml.

TABLE 1. Susceptibility of H. influenzae isolates to cefatrizine, ampicillin, and chloramphenicol

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameter</th>
<th>MIC (88 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone size (mm)</td>
<td>No. of isolates</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>0.063</td>
</tr>
<tr>
<td>CEF</td>
<td>MIC</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>1</td>
</tr>
<tr>
<td>AMP</td>
<td>MIC</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>6</td>
</tr>
<tr>
<td>CHL</td>
<td>MIC</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>3</td>
</tr>
</tbody>
</table>

a CEF, Cefatrizine; AMP, ampicillin; CHL, chloramphenicol.

b Antibiotic concentration (micrograms per milliliter).

c MBC, Minimal bactericidal concentration.
The activity of cefatrizine against *H. influenzae* appears to be comparable in vitro to that of ampicillin.

Concentrations of cefatrizine in serum have been measured in 11 children with cervical lymphadenitis. Concentrations of this antibiotic peaked at 1 h after the initial dose (mean, 4.72 μg/ml; range, 3.5 to 7.6 μg/ml). Concentrations at 2 h were virtually identical to those obtained 1 h after a dose. At 6 h (immediately prior to a second dose) the concentration in serum was 1.19 μg/ml (mean), and a range of 0.3 to 3.0 μg/ml was noted.

**DISCUSSION**

Only six isolates of *H. influenzae* were resistant to cefatrizine by the disk susceptibility test method. Thus, the activity of cefatrizine did not appear to differ significantly from that of ampicillin. All four ampicillin-resistant *H. influenzae* strains were susceptible to cefatrizine. These data are in agreement with information reported by most other investigators who have tested the susceptibility of smaller numbers of *H. influenzae* isolates (3, 5, 7). In one report, however, Actor and associates (1) reported that 6.3 to 12.5 μg of cefatrizine per ml was required to inhibit 90% of the *H. influenzae* isolates tested by them. The number of isolates of *H. influenzae* utilized in their study was not stated. The activity of cefatrizine against *H. influenzae* was not compared with that of ampicillin or chloramphenicol, drugs frequently used in the treatment of *H. influenzae* infections in clinical practice.

Concentrations of cefatrizine in serum after administration of a 10-µg/kg dose orally peaked at values that were twofold in excess of the MIC of 84 of the 88 *H. influenzae* isolates tested in this study. These data, coupled with the in vitro studies described above, document that it is possible to achieve concentrations of cefatrizine in serum that may prove efficacious in the treatment of *H. influenzae* infections.

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**LITERATURE CITED**