Comparative In Vitro Bactericidal Activity of Cefonicid, Ceftizoxime, and Penicillin Against Group B Streptococci

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Penicillin G and ampicillin have remained the drugs of first choice in infections due to group B streptococci (GBS) because of low minimal inhibitory and bactericidal concentrations (MICs, MBCs) reported in most studies (3). However, investigators have been prompted to examine non-penicillin regimens for in vitro inhibitory and bactericidal efficacy for several reasons: (i) high morbidity and mortality rates of GBS infections, especially in neonates (2); (ii) increasing reports of relapse or recurrence of GBS infection despite penicillin therapy (4); (iii) reports of GBS strains relatively resistant or tolerant to the killing action of penicillin (1, 8); and (iv) limited data on alternative (non-penicillin) bactericidal agents for GBS, especially for patients who are allergic to penicillin or who develop penicillin end-organ toxicity (e.g., nephropathy).

We have examined the in vitro inhibitory and bactericidal activity of two new cephalosporins, cefonicid (first generation) and ceftizoxime (third generation), against 108 blood and spinal fluid GBS isolates and compared these activities with those of penicillin G.

The bacterial strains used in this study were identified as GBS according to Lancefield’s criteria (9). The GBS were isolates of the Harbor-UCLA Medical Center, the University of Minnesota Hospitals, the University of Alabama Medical Center, and the Charity Hospital of New Orleans, Louisiana, between 1976 and 1980. There were no duplicate isolates from the same patient. Aliquots of logarithmic-phase cultures of each strain in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) were stored at −70°C until susceptibility testing was performed.

A late-logarithmic-phase culture of each GBS isolate was prepared in Mueller-Hinton broth as previously described (7, 8) at $\sim 2 \times 10^5$ colony-forming units per ml. Potassium penicillin G (Bristol Laboratories, Syracuse, N.Y.), and ceftizoxime (Smith, Kline & French Laboratories, Philadelphia, Pa.) were reconstituted to concentrations of 10,000 μg/ml and stored in aliquots at −70°C until the susceptibility testing was performed (within 4 weeks of freezing).

MICs and MBCs of the 108 GBS isolates to penicillin, cefonicid, and ceftizoxime were determined in duplicate by the microtiter broth-dilution technique; a GBS control strain with previously determined MICs and MBCs was included with each run as a check on reproducibility. Each antibiotic agent to be tested was thawed immediately before use, and serial twofold dilutions were made in Mueller-Hinton broth in the microtiter wells. For cefonicid and ceftizoxime, the final drug concentration in the wells was 0.02 to 25 μg/ml; for penicillin this range was 0.005 to 6.25 μg/ml. These ranges were selected to encompass drug concentrations which are readily achievable in serum after administration in conventional dose regimens. The final GBS inoculum into each antibiotic-containing microtiter well was $\sim 10^4$ colony-forming units. The plates were incubated at 37°C for 24 h. The MIC was defined as the lowest antibiotic concentration inhibiting visible turbidity. The MBC was determined by subculturing 25 μl from each microtiter well onto sterile, antibiotic-free blood agar plates with a micropette. These plates were incubated at 37°C for 24 h. The MBC was defined as the lowest antibiotic concentration causing $\geq 99.9\%$ killing of the original inoculum (two colonies or less).

Comparative statistical assessments of the three antimicrobial agents was performed by Student’s t test for unpaired samples.

The inhibitory and bactericidal susceptibilities of these 108 GBS strains to penicillin, cefonicid, and ceftizoxime are summarized in Table 1.
Penicillin G was the most active inhibitory and bactericidal agent. All 108 strains were inhibited by low penicillin concentrations (≤0.08 μg/ml). In addition, 100% of strains were killed by ≤0.78 μg of penicillin per ml. The geometric mean MIC and MBC for penicillin were 0.04 ± 0.02 and 0.07 ± 0.02 μg/ml, respectively. However, 18% of GBS strains required penicillin MBCs between 0.1 and 1 μg/ml, the range of relative penicillin resistance for cerebrospinal fluid streptococcal isolates (6). Four of the 108 strains were “tolerant” to the killing action of penicillin, with MBC/MIC ratios of ≥16:1 (7, 8).

Ceftizoxime was a less active inhibitory and bactericidal agent than penicillin G (P < 0.0005), but was significantly more active than cefonicid (P < 0.0005 and P < 0.025 for inhibitory and bactericidal activities, respectively). Of the 108 GBS strains, 103 (95%) had ceftizoxime MICs of ≤0.02 μg/ml, versus only 2/108 isolates with cefonicid MICs of <0.02 μg/ml. Ceftizoxime was also a significantly more active bactericidal agent than cefonicid, with 72% of GBS strains having ceftizoxime MBCs of ≤0.39 μg/ml (versus 22% of cefonicid MBCs at ≤0.39 μg/ml). The geometric mean MIC and MBC for ceftizoxime were 0.38 ± 0.02 and 0.85 ± 0.02 μg/ml, respectively. In contrast, the geometric mean MIC and MBC for cefonicid were 0.11 ± 0.02 and 0.32 ± 0.02 μg/ml, respectively. Of the GBS strains, 16% and 10%, respectively, were tolerant to the killing action of ceftizoxime and cefonicid.

There is limited published information on the in vitro and in vivo bactericidal efficacy of non-penicillin antimicrobial agents against the GBS. Such data are vital in situations requiring bactericidal effect (e.g., meningitis) in which penicillin regimens have either failed, caused major allergies, or been associated with end-organ toxicity.

Cefonicid and ceftizoxime are candidate agents for in vitro evaluation against GBS for several reasons (10, 12): (i) both have excellent in vitro activity against beta-hemolytic streptococci, especially group A; (ii) both achieve peak serum levels of ≥100 μg/ml after 1-g intravenous bolus doses in humans; (iii) both have relatively prolonged serum half-lives versus other β-lactams (cefonicid, 4.8 h; ceftizoxime, 1.3 h); and (iv) ceftizoxime penetrates inflamed meninges well (Don Parks, personal communication). This is particularly applicable to GBS meningitis, the most common clinical GBS syndrome. Ceftizoxime resembles other new β-lactams in this regard, such as moxalactam, cefoperazone, rocephin, and cefotaxime (11).

The current study demonstrated that cefonicid is a clearly inferior in vitro bactericidal agent against the GBS as compared to both penicillin and ceftizoxime. Also, ceftizoxime appears to have significant in vitro bactericidal activity against most GBS strains. There are no other reported series of the in vitro bactericidal efficacy of the newer cephalosporin agents against large numbers of GBS strains as is currently presented. However, ceftizoxime appears to have MBCs against nontolerant GBS comparable to those of cefotaxime, cefoperazone, and rocephin and superior to that of moxalactam (11). In a study of only nine GBS clinical isolates, Shaad et al. (11) reported an MBC of cefoperazone and rocephin for 90% of isolates of 0.25 μg/ml, whereas for cefotaxime it was 0.12 μg/ml. This compares favorably to the ceftizoxime MBC for 90% of isolates of 0.48 μg/ml for the 92 nontolerant GBS strains in the present study.

Considering the attractive pharmacokinetic properties of ceftizoxime, this agent warrants further evaluation in in vivo animal models of severe GBS infection due to susceptible strains (i.e., neonatal meningitis [5] and endocarditis). The finding in our study that 16% of the 108 GBS isolates tested were tolerant to the killing action of ceftizoxime may limit this agent’s utility. Such tolerance should be carefully tested for when considering use of the newer cephalosporin agents in serious GBS infections.

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**TABLE 1. In vitro susceptibilities of 108 GBS isolates to cefonicid, ceftizoxime, and penicillin G**

<table>
<thead>
<tr>
<th>Agent</th>
<th>MICs (μg/ml) Range</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>MBCs (μg/ml) Range</th>
<th>MBC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MBC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefonicid</td>
<td>0.04-3.12</td>
<td>0.32</td>
<td>0.67</td>
<td>0.19-6.25</td>
<td>0.56</td>
<td>1.49</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>0.04-0.78</td>
<td>0.08</td>
<td>0.17</td>
<td>0.09-3.12</td>
<td>0.13</td>
<td>0.48</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.04-0.08</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04-0.39</td>
<td>0.05</td>
<td>0.15</td>
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<table>
<thead>
<tr>
<th>Nontolerant strains</th>
<th>Tolerant strains&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>MBC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MBC&lt;sub&gt;90&lt;/sub&gt;</td>
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<td>---------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>6.25-25</td>
<td>10.4</td>
</tr>
<tr>
<td>1.56-12.5</td>
<td>1.8</td>
</tr>
<tr>
<td>0.78</td>
<td>0.78</td>
</tr>
</tbody>
</table>

<sup>a</sup> Subscripts indicate percentage of strains inhibited or killed.

<sup>b</sup> Cefonicid, 98 nontolerant and 10 tolerant strains; ceftizoxime, 92 nontolerant and 16 tolerant strains; penicillin, 104 nontolerant and 4 tolerant strains.
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LITERATURE CITED