In Vitro Activity of Ceftizoxime Against Bacteroides fragilis: Comparison with Benzylpenicillin, Cephalothin, and Cefoxitin

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Minimum inhibitory concentrations of ceftizoxime for seven clinical isolates of Bacteroides fragilis were found to be markedly affected by inoculum size; the very low minimum inhibitory concentration values (≤0.25 μg/ml) obtained in tests with most strains were not sustained when the inoculum was raised 100-fold. Benzylpenicillin and cephalothin were also affected by inoculum size, but cefoxitin was not. Antibiotic assays showed that the strains inactivated ceftizoxime and cephalothin completely, benzylpenicillin partially, and cefoxitin not at all. Morphological investigations revealed that populations of B. fragilis responded differently to each of the four β-lactam agents during the early stages of the encounter between B. fragilis and the antibiotic. Benzylpenicillin and cefoxitin induced spheroplast formation at lower concentrations than did ceftizoxime. Cephalothin evoked a novel morphological response that was not seen with other β-lactam agents.

In the past few years, a number of β-lactam compounds have been described which combine exceptional antibacterial activity with stability against most β-lactamases. Among the most active of such recently described compounds is ceftizoxime (1, 2, 4).

Ceftizoxime would seem to have potential for use as a single agent in the therapy of polymicrobial infections involving Bacteroides fragilis since it has a much broader spectrum than non-β-lactam antianaerobe agents and is much more active than other β-lactam antibiotics, including cefoxitin, when tested against Bacteroides species in conventional minimum inhibitory concentration (MIC) tests. However, hydrolysis of ceftizoxime by a purified β-lactamase from B. fragilis has been reported, in contrast to the absolute stability of cefoxitin (6).

In this study we have compared the activities of ceftizoxime, cefoxitin, benzylpenicillin, and cephalothin against clinical isolates of B. fragilis in conventional MIC tests with different inocula and tested the ability of the bacteria to degrade these β-lactam compounds. We have also investigated the morphological response of B. fragilis to the four β-lactam antibiotics.

MATERIALS AND METHODS

Antibiotics. Ceftizoxime (sodium salt) was provided by Fujisawa Pharmaceutical Co., Ltd.; cefoxitin was provided by Merck Sharp & Dohme, Ltd.; cephalothin was provided by Eli Lilly & Co., Ltd. Benzylpenicillin was a standard pharmaceutical preparation obtained from the hospital pharmacy. Suitable concentrations of antibiotic were freshly prepared in sterile distilled water as required.

Culture medium. Brain heart infusion broth, supplemented with hemin (5 μg/ml) and menadione (1 μg/ml) (BHI-S), was used throughout. Agar (1.5%) and lysed horse blood (5%) were added for agar dilution MIC titrations. All culture media were prereduced by incubation overnight in a mixture of 85% nitrogen, 5% carbon dioxide, and 10% hydrogen.

Bacteria. Seven strains of B. fragilis were freshly isolated from clinical samples submitted to the diagnostic microbiology laboratory. Organisms were identified by procedures recommended by Holdeman and Moore (3).

Antibiotic titrations. Antibiotic MICs were estimated by using an agar dilution technique in which serial doubling dilutions of antibiotic were prepared in BHI-S-lysed blood agar. Overnight BHI-S broth cultures of bacteria were diluted 1/10 and 1/1,000 in fresh broth, and 1 drop of each dilution was applied to the antibiotic-containing plates by using an automatic multipoint inoculation device (Denly-Tech Ltd.). The inoculator delivered approximately 10^5 and 10^7 bacteria, respectively, from the two dilutions of bacteria used.

Plates were incubated anaerobically for 48 h, and the endpoint was taken as the highest dilution causing complete inhibition of growth. In titrations involving the higher inoculum, a faint haze of growth was sometimes seen which was ignored in assessing the endpoint.

Antibiotic stability assays. Volumes (20 ml each) of BHI-S broth were inoculated with 1 ml of an overnight broth culture of B. fragilis to give an initial inoculum of ca. 5 × 10^7 bacteria per ml. The tubes
were incubated in a modified version of the multichannel bacterial growth monitoring device described by Mackintosh et al. (5). When bacterial growth had raised the opacity to a level of 50% of that of a fully grown culture (equivalent to a viable count of ca. 5 × 10⁶ bacteria per ml), sufficient antibiotic was added to achieve the desired concentration, and incubation in the turbidimeter was continued overnight.

After overnight incubation, cultures were centrifuged at 3,000 rpm for 20 min. Supernatants were removed and assayed by using a well-diffusion technique with Staphylococcus aureus NCTC 6571 or Escherichia coli NCTC 10418 as the indicator organism. Standard concentrations of antibiotic in BHI-S broth, but without bacteria, were exposed to identical conditions of incubation and assayed in parallel to take into account any natural instability of the compounds.

**Microscopy.** Antibiotic-induced morphological changes in bacteria were observed by interference-contrast microscopy of samples taken from turbidimetric experiments 60 min after addition of antibiotic.

**RESULTS**

**MICs.** When tested against a low (10³) bacterial inoculum, benzylpenicillin, cephalothin, and cefoxitin exhibited similar activity against six of the seven *B. fragilis* strains, with benzylpenicillin appearing marginally to be the most active of the three (Table 1). Cefoxitoxime was substantially more active against these strains, with a concentration of 0.25 μg/ml inhibiting all six. The seventh strain was resistant to benzylpenicillin and cephalothin and exhibited an elevated MIC value for cefotizoxime (Table 1).

When the inoculum was raised 100-fold, MIC values of benzylpenicillin, cephalothin, and cefotizoxime were all markedly increased. Cefoxitoxime was particularly dramatically affected by the increase in inoculum size, whereas cefoxitin was hardly affected at all (Table 1).

**Antibiotic assay.** No antibacterial activity was detected after overnight incubation of cultures of *B. fragilis* with cephalothin or cefotizoxime. Benzylpenicillin concentrations fell during the overnight exposure period, but antibacterial activity was still detectable. No loss of activity of cefoxitin was detected. Representative results for one of the *B. fragilis* strains are shown in Fig. 1.

**Morphological response.** The morphological changes induced in *B. fragilis* strain 1 by various concentrations of the four β-lactam agents are shown in Fig. 2. Benzylpenicillin, cefoxitin, and cefotizoxime each evoked filamentation (Fig. 2B) at a low concentration and spheroplast formation (Fig. 2C) at higher concentrations. Cephalothin induced a different response into that affected cells assumed a bizarre, bloated appearance (Fig. 2D), whereas filamentation and spheroplast formation were not observed. The concentration ranges over which the various morphological changes were observed are shown diagrammatically in Fig. 3. Cefoxitin was able to evoke spheroplast formation at lower concentrations than was benzylpenicillin, and cefotizoxime was least active in this respect. Essentially similar results were obtained with three other strains examined in this way.

**DISCUSSION**

Although cefotizoxime showed excellent activity against *B. fragilis* when tested against low bacterial inocula, this activity was not maintained against the larger bacterial numbers which may be encountered in infection. A similar inoculum effect has been noted with the closely related compound cefotaxime (8) and attributed to β-lactamase activity on the basis of protection afforded by the β-lactamase inhibitor, clavulanic acid (8).

In this study, stability tests have shown that the inoculum effect with cefotizoxime is associated with antibiotic destruction by the bacteria and, disappointingly, these new compounds, which otherwise exhibit outstanding activity and stability, appear to share the general susceptibility of earlier cephalosporins to *B. fragilis* β-lactamase (7). However, as previously described (7) and confirmed here, the cephamycin antibiotic, cefoxitin, appears fully stable to enzymatic attack by *B. fragilis*.

When the activity of cefotizoxime was compared with those of other β-lactam compounds in terms of morphological criteria, it was found that the new cephalosporin was less active than benzylpenicillin or cefoxitin in inducing the cell wall-deficient spheroplasts which lethal concentrations of β-lactam antibiotics typically induce in susceptible gram-negative rods. However, cef-

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**TABLE 1. MICs of cefotizoxime, benzylpenicillin, cephalothin, and cefoxitin for seven strains of *B. fragilis***

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cefotizoxime</th>
<th>Benzylpenicillin</th>
<th>Cephalothin</th>
<th>Cefoxitin</th>
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<tbody>
<tr>
<td></td>
<td>10⁶</td>
<td>10⁵</td>
<td>10⁴</td>
<td>10³</td>
</tr>
<tr>
<td>1</td>
<td>≤0.25</td>
<td>0.25</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
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<td>16</td>
<td>12</td>
</tr>
<tr>
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<td>0.25</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>≤0.25</td>
<td>0.25</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
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<td>0.25</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
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<td>0.25</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>≤0.25</td>
<td>0.25</td>
<td>4</td>
<td>32</td>
</tr>
</tbody>
</table>

* a Inoculum in colony-forming units per plate.
FIG. 1. Stability of benzylpenicillin, cephalothin, cefoxitin, and ceftizoxime during overnight incubation in the presence of B. fragilis (○) and in the absence of bacteria (○).

FIG. 2. Morphological response of B. fragilis after a 1-h exposure to β-lactam antibiotics. (A) No antibiotic; (B) ceftizoxime at 16 μg/ml; (C) benzylpenicillin at 16 μg/ml; (D) cephalothin at 128 μg/ml.
ACTIVITY OF CEFTIZOXIME AGAINST B. FRAGILIS

thin produced a novel response in B. fragilis which was not seen with the other compounds. The significance of this is not known.

There are no clinical reports yet of the efficacy of ceftizoxime in the treatment of infection, but these results do not encourage the view that this new cephalosporin will be suitable for use in infections involving B. fragilis.

LITERATURE CITED