In Vitro Activities of Cefoperazone and Sulbactam Singly and in Combination against Cefoperazone-Resistant Members of the Family Enterobacteriaceae and Nonfermenters

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Among 28,000 isolates of the family Enterobacteriaceae and nonfermenters isolated at multiple medical centers, 1,084 (4%) were resistant to cefoperazone (MIC, ≥64 μg/ml) and 1,711 (6%) exhibited cefoperazone MICs of 2 to 32 μg/ml. Ninety-six percent of these 2,795 isolates produced β-lactamase, as determined by the nitrocefin test. Sulbactam alone (8 μg/ml) was inactive against 99.6% of the isolates other than Acinetobacter calcoaceticus and Pseudomonas cepacia. Sulbactam enhanced the activity of cefoperazone against 56% of the isolates of the family Enterobacteriaceae and 44% of the nonfermenters. In the presence of sulbactam concentrations of ≤8 μg/ml, 65% of the cefoperazone-resistant isolates had reductions in cefoperazone MICs of ≥2 log₂ dilution steps and were susceptible to ≤32 μg/ml. Antagonism was not observed.

Cefoperazone is an extended-spectrum cephalosporin; most staphylococci, streptococci, members of the family Enterobacteriaceae, nonfermenters, and anaerobes are susceptible to concentrations observed in vivo with recommended dosing regimens (1, 7). As a piperazinyl derivative, it has structural characteristics similar to those of the amnopenicillin piperaicillin and is more active against Pseudomonas aeruginosa and enterococci than the iminopenicillins. Sulbactam (penicillanic acid sulfone; CP-45,899) is a derivative of the basic penicillin nucleus (2). Sulbactam inhibits a broad spectrum of β-lactamases produced by gram-positive and gram-negative organisms (4, 5, 9, 10, 14, 16–19), but its intrinsic antibacterial activity is limited.

In this study, investigators at medical centers in diverse geographic locations of the United States screened recent clinical isolates and determined their susceptibilities to cefoperazone and sulbactam, singly and in combination.

Laboratories from participating institutions isolated bacterial pathogens from clinical material and tested approximately 28,000 isolates of the family Enterobacteriaceae and nonfermenters for susceptibility to cefoperazone in vitro (12). Each isolate was identified to the species level by standard methods (11), and β-lactamase production was determined by a rapid chromogenic cephalosporin (nitrocefin) test (Cefinase; BBL Microbiology Systems, Cockeysville, Md.). No more than one isolate of the same species from any given patient was included.

Among the 28,000 isolates, 2,795 with cefoperazone MICs of ≥2 μg/ml were tested for their susceptibilities to cefoperazone, sulbactam, and the combination of cefoperazone-sulbactam in a checkerboard microdilution test. Eighty-seven microdilution plates manufactured specifically for this study (Prepared Media Laboratories, Tualatin, Oreg.) were delivered frozen to participating laboratories and stored at −20°C until use. Each plate contained wells with cefoperazone in doubling dilutions over the concentration range of 1.0 to 256 μg/ml, sulbactam in doubling dilutions over the range of 0.25 to 16 μg/ml, and all possible combinations of cefoperazone and sulbactam within those ranges. Antibiotic dilutions were made in cation-supplemented Mueller-Hinton broth. After thawing, the plates were inoculated with disposable inoculators so that the final inoculum in each well was 1 × 10⁵ to 2 × 10⁵ CFU/ml. Microdilution plates were incubated at 35°C for 15 to 18 h, and MIC determinations were made with the aid of a backlit MIC panel reader; results were recorded on a standardized form for computer entry. For quality control, the susceptibility of Acinetobacter anitratus S2 (ATCC 43498) cefoperazone MIC, 16 to 64 μg/ml; sulbactam MIC, 0.5 to 2 μg/ml) was tested daily.

Isolates were grouped according to cefoperazone MIC. One group included organisms for which MICs were ≥64 μg/ml, and the second group included organisms for which MICs were in the range of 2 to 32 μg/ml. Isolates with cefoperazone MICs of ≥64 μg/ml were considered resistant. Enhancement was defined as a reduction in the cefoperazone MIC by at least 2 log₂ dilution steps in the presence of ≤8 μg of sulbactam per ml compared with the MIC of cefoperazone alone. For the cefoperazone-resistant strains (MICs, ≥64 μg/ml), the percentage of strains for which there was enhancement plus conversion from the resistant inter-
TABLE 1. Sublactam-enhanced activity of cefoperazone and geometric mean cefoperazone MICs in the presence or absence of sublactam for 2,795 clinical isolates for which cefoperazone MICs were \( \approx 2 \mu g/ml \)

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains tested</th>
<th>Enhance-ment observed (% of strains)</th>
<th>Geometric mean cefoperazone MIC (( \mu g/ml )):</th>
<th>Activity index*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without sublactam</td>
<td>With sublactam</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus</td>
<td>223</td>
<td>36</td>
<td>45.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>375</td>
<td>99</td>
<td>30.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>36</td>
<td>75</td>
<td>45.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>76</td>
<td>68</td>
<td>16.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>246</td>
<td>68</td>
<td>38.5</td>
<td>5.2</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>103</td>
<td>86</td>
<td>40.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>139</td>
<td>60</td>
<td>71.8</td>
<td>19.3</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>219</td>
<td>50</td>
<td>59.7</td>
<td>16.9</td>
</tr>
<tr>
<td>Citrobacter diversus</td>
<td>18</td>
<td>50</td>
<td>50.8</td>
<td>14.8</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>24</td>
<td>50</td>
<td>5.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>294</td>
<td>44</td>
<td>36.5</td>
<td>12.1</td>
</tr>
<tr>
<td>Klebsiella oxytica</td>
<td>100</td>
<td>23</td>
<td>18.4</td>
<td>7.9</td>
</tr>
<tr>
<td>Xanthomonas malophilia</td>
<td>33</td>
<td>27</td>
<td>28.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Pseudomonas cepacia</td>
<td>40</td>
<td>27</td>
<td>16.0</td>
<td>8.5</td>
</tr>
<tr>
<td>Providencia stuartii</td>
<td>194</td>
<td>36</td>
<td>6.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>520</td>
<td>10</td>
<td>16.9</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* Ratio of geometric mean MICs of cefoperazone to geometric mean MICs of the cefoperazone component of cefoperazone and sublactam in combination.

1 Sublactam was present at 8 \( \mu g/ml \).

2 Sublactam alone (\( \geq 8 \mu g/ml \)) inhibited 339 (95%) of these strains.

3 Sublactam alone (\( \geq 8 \mu g/ml \)) inhibited 10 (30%) of these strains.

The frequency with which sublactam enhanced the activity of cefoperazone and the geometric mean MICs of cefoperazone without and with sublactam for the 2,795 study organisms are given in Table 1. Overall, sublactam enhanced the activity of cefoperazone against 56% of the Enterobacteriaceae and 44% of the nonfermenters. No instances of antagonism were observed.

Table 2 shows the frequency of enhancement by group; for the 1,084 cefoperazone-resistant isolates (MICs, \( \geq 64 \mu g/ml \)), the percentage of the isolates that were converted from the resistant interpretive category to the susceptible category by the addition of sublactam is included in the definition of enhancement. Enhancement was dependent on the concentration of sublactam, and the effect of any given concentration of sublactam varied with the bacterial species, as illustrated in Fig. 1.

Twenty-five of the cefoperazone-resistant isolates did not produce detectable \( \beta \)-lactamase, as follows: Acinetobacter calcoaceticus \( (n = 8) \); Escherichia coli \( (n = 4) \); Pseudomonas aeruginosa \( (n = 3) \); Xanthomonas malophilia \( (n = 3) \); Citrobacter freundii \( (n = 2) \); Providencia stuartii \( (n = 2) \); and Klebsiella pneumoniae, Klebsiella oxytoca, and Enterobacter cloacae \( (n = 1) \) each. Nevertheless, the combination exhibited enhanced activity for 12 of the 18 (67%) isolates in this subgroup that were not susceptible to sublactam alone.

Cefoperazone is very active against most strains of the family Enterobacteriaceae and Pseudomonas aeruginosa (1, 5, 6, 7, 15); median MICs are typically \( \leq 4 \) and 4 to 8 \( \mu g/ml \), respectively, and a susceptibility breakpoint of \( \leq 32 \mu g/ml \) is generally accepted. Many bacterial species, those that are both susceptible and resistant to cefoperazone, produce \( \beta \)-lactamase enzymes (3-5, 8, 13, 15). Although cefoperazone is vulnerable to the hydrolytic activity of some of these enzymes, they are only partially responsible for cefoperazone resistance (3, 13), and other mechanisms may contribute to the resistance phenotype. The concomitant administration of a \( \beta \)-lactamase inhibitor such as sublactam would be expected to enhance the activity of cefoperazone against organisms if the following four conditions were present: (i) the organisms produced \( \beta \)-lactamase, (ii) the enzyme hydrolyzed the active antibiotic (cefoperazone), (iii) the primary
mechanism of resistance was due to β-lactamase production, and (iv) the inhibitor (sulbactam) inactivated the enzyme. The inhibitor could also enhance the activity of cefoperazone if it had significant intrinsic activity against the organisms, as is the case with Neisseria gonorrhoeae, Acinetobacter calcoaceticus, and Pseudomonas cepacia, or if it had a direct action (complementary to β-lactamase inhibition) on the penicillin-binding proteins of inhibitor-resistant organisms. In this study, the net effect of these various interactions on organisms from diverse geographic locations was determined with a large sample of isolates resistant to cefoperazone or for which cefoperazone MICs were 2 to 32
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VOL. 34, distribution of MICs might vary with the testing method. For example, if a broth macrodilution method had been used, the greater total number of organisms in a given inoculum would increase the chance of detecting an elevated MIC for a small portion of the population tested.

Sublactam-mediated enhancement of the activity of cefoperazone was observed more frequently against members of the family Enterobacteriaceae than against Pseudomonas species. It is likely that many of the former produced β-lactamase that was inhibited by sublactam at the concentrations tested, whereas the latter did not. However, β-lactamase production, as measured by the nitrocefin test, was not a prerequisite for enhancement. The apparent synergistic effect of cefoperazone and sublactam against Acinetobacter calcoaceticus and some strains of Pseudomonas cepacia, organisms which are typically more resistant to cefoperazone than members of the family Enterobacteriaceae and Pseudomonas aeruginosa, were, more likely due to the intrinsic activity of sublactam than to β-lactamase inhibition. In addition, sublactam increased the observed activity of cefoperazone against some of the β-lactamase-negative isolates, although it did not potentiate activity against all β-lactamase-positive isolates. This suggests that alternate mechanisms, such as porin restrictions or alterations of the cefoperazone or sublactam target(s), contribute to cefoperazone resistance in some organisms. The reduction in the MIC observed for the small number of cefoperazone-resistant, β-lactamase-negative isolates may be the result of classical synergy of the two β-lactams or may reflect the limits of nitrocefin in detecting β-lactamase(s). Induction of inapparent chromosomal β-lactamases was not attempted in this study.

The data presented here suggest that sublactam increases the activity of cefoperazone against the Enterobacteriaceae and nonfermenters to various degrees in a species- and concentration-dependent manner and converts many cefoperazone-resistant strains into the susceptible range.

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LITERATURE CITED


