Therapy with Cefoperazone plus Sulbactam against Disseminated Infection Due to Cefoperazone-Resistant Pseudomonas aeruginosa and Escherichia coli in Granulocytopenic Mice

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Using a granulocytopenic murine model, we evaluated the efficacy of cefoperazone plus sulbactam against disseminated infection due to isolates of β-lactamase-producing, cefoperazone-resistant (MIC, ≥50 μg/ml) Escherichia coli and Pseudomonas aeruginosa. Both isolates were susceptible in vitro to cefoperazone plus sulbactam (MIC, ≤6.3 μg/ml). Mice rendered granulocytopenic with cyclophosphamide were divided into three groups: group A—infected, untreated mice (controls); group B—injected, cefoperazone-treated mice (700 mg/kg of body weight); and group C—infected, cefoperazone-plus-sulbactam-treated mice (700 mg plus 350 mg). In the E. coli experiment, survival rates in groups A, B, and C were 25, 46, and 73%, respectively. In the experiment with P. aeruginosa, survival rates in groups A, B, and C were 0, 10, and 50%, respectively (P < 0.001). Highly significant differences also were noted for colony counts in the blood, liver, and spleen of group C mice versus group A or B mice in both experiments. Thus, cefoperazone plus sulbactam appears to be a promising combination for the treatment of infections due to certain cefoperazone-resistant gram-negative bacilli, including P. aeruginosa.

Gram-negative bacteria of the family Enterobacteriaceae and Pseudomonas aeruginosa are important pathogens during chemotherapy-induced granulocytopenia in cancer patients. These microorganisms are generally susceptible to beta-lactam drugs, such as cefoperazone, ceftazidime, and imipenem, that are commonly used as initial therapy during febrile neutropenia. Occasionally, however, organisms resistant to broad-spectrum beta-lactam agents are encountered (2), and inappropriate initial therapy in such instances is likely to result in a poor outcome. As antimicrobial resistance among bacteria becomes more prevalent, there is an ever-increasing need for more effective antimicrobial agents. Bacterial β-lactamase production is the most common mechanism of resistance among gram-negative bacteria. Sulbactam is a semisynthetic beta-lactam sulfone compound that can irreversibly bind to clinically prevalent chromosome- and plasmid-mediated β-lactamases, thus rendering these enzymes ineffective (8). The combination of cefoperazone and sulbactam has been shown to be remarkably active in vitro against cefoperazone-resistant gram-negative bacteria, including P. aeruginosa (2, 3, 13). We have extended this work by using a murine model to evaluate the in vivo efficacy of cefoperazone plus sulbactam in the treatment of disseminated infection due to clinical isolates of cefoperazone-resistant Escherichia coli and P. aeruginosa.

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MATERIALS AND METHODS

Organisms. Two cefoperazone-resistant clinical isolates, one each of E. coli and P. aeruginosa, were selected for the study. β-Lactamase production was detected by use of nitrocefin discs. A color change within 30 min indicated the presence of β-lactamase.

Antibiotics and susceptibility testing. Cefoperazone and sulbactam were kindly provided by Pfizer (Roerig) Laboratories, New York, N.Y. Stock solutions were prepared in accordance with the manufacturer's directions and frozen at −70°C until further use. Sulbactam and cefoperazone were combined at a ratio of 1:2. Other antibiotics tested were cefotaxime, tobramycin, imipenem, and ciprofloxacin.

MICs were determined by the broth microdilution method with 10^6 CFU of organisms inoculated into supplemented Mueller-Hinton broth per ml in microtiter plates. The plates were incubated at 35 to 37°C for 24 h, and the MIC was defined as the lowest concentration of drug to inhibit visible growth. MBCs were determined by inoculating a 10-μl aliquot from each microtiter well onto plates containing 40 ml of Trypticase soy agar. The plates were incubated at 35 to 37°C for 24 h, and the MBC was defined as the lowest concentration of drug to prevent bacterial growth.

Animal model. Female ICR mice weighing 20 to 22 g were rendered granulocytopenic by three intraperitoneal injections of 100 mg of cyclophosphamide per kg of body weight given every other day. Peripheral leukocyte counts in pooled blood samples were checked daily. Granulocytopenia was defined as an absolute granulocyte count of <500/mm³. All granulocytopenic mice were kept in separate cages.

Infection. The cefoperazone-resistant isolate of E. coli was grown on a cell-culture rotator to the log phase in Mueller-Hinton broth at 37°C for 4 h. The broth was centrifuged at 17,500 × g for 10 min, and the centrifuged organisms were resuspended in normal saline. Using a spectrophotometer, we adjusted the inoculum chosen for the experiment to 4 × 10^8 CFU/ml. (Preliminary experiments were done to determine the optimal size of the inoculum of E. coli adequate to...
cause death in >75% of untreated animals within 72 h of infection.) The inoculum of *E. coli* was introduced intraperitoneally into the mice on the second day of neutropenia.

An identical experiment was carried out with cefoperazone-resistant *P. aeruginosa* as the infecting organism. The inoculum used, after testing in preliminary experiments, was $4.5 \times 10^7$ CFU/ml.

**Therapy.** Antibiotic treatment was begun within 2 h of infection. The agents used were cefoperazone at 700 mg/kg of body weight and cefoperazone plus sulbactam at 700 mg plus 350 mg. Preliminary experiments had been done with mice to determine the dosing schedules for cefoperazone and cefoperazone plus sulbactam that would produce peak levels comparable to those in humans. The mice were divided into three groups: group A—infected, untreated mice (controls); group B—infected mice treated with cefoperazone; and group C—infected mice treated with cefoperazone plus sulbactam. The drugs were given subcutaneously every 8 h for 3 days. Control mice were given subcutaneous injections of normal saline as a placebo.

**Assessment of responses.** Serum antibiotic levels in blood specimens drawn after the third dose of antibiotic were determined. High-pressure liquid chromatography was used for the drug assay (Biopharmaceutical Reference Laboratory, Houston, Tex.).

Each group was monitored daily for number of survivors. On the day following the completion of therapy, all surviving mice were sacrificed and autopsied. Also, mice that died during the experiment were autopsied. Blood was obtained for quantitative culturing at the time of autopsy. The liver and spleen were removed by use of an aseptic technique, rinsed in sterile saline, and homogenized. For quantitative culturing, a 0.1-ml sample from the homogenates of each of the organs was serially diluted in normal saline, plated in triplicate on Trypticase soy agar, and incubated for 24 h at 37°C.

**Statistical methods.** Kaplan-Meier survival curves were constructed for the three groups and compared by use of the log-rank chi-square test. Colony counts were expressed as base 10 logarithmic transformations. Sterile cultures were ranked below positive-count values, and colony count ranks were compared by use of the Kruskal-Wallis test with corrections for ties. Multiple pairwise comparisons of the groups were made by use of Dunn's nonparametric procedure with corrections for ties. *P* values of <0.05 were considered statistically significant.

**RESULTS**

**Antibiotic susceptibility and β-lactamase production.** The *E. coli* and *P. aeruginosa* isolates examined were cefoperazone resistant (MIC, ≥50 μg/ml) but susceptible to the combination of cefoperazone and sulbactam. The MICs and MBCs of cefoperazone in the combination were 3.2 and 6.3 μg/ml for *E. coli* and 6.3 and 12.5 μg/ml for *P. aeruginosa*. The respective MICs of cefotaxime, tobramycin, imipenem, and ciprofloxacin were 0.1, 100, 0.04, and <0.05 μg/ml for *E. coli* and >100, 6.2, 0.78, and 0.10 μg/ml for *P. aeruginosa*. Both isolates produced β-lactamase, as detected by use of nitrocefin discs.

**Animal model.** There were a total of 42 mice (12 controls, 15 cefoperazone-treated, and 15 cefoperazone-plus-sulbactam-treated mice) in the experiment with *E. coli* and 56 mice (16 controls, 20 cefoperazone-treated, and 20 cefoperazone-plus-sulbactam-treated mice) in the experiment with *P. aeruginosa*.

![Fig. 1](http://aac.asm.org/)

**FIG. 1.** Survival curves for mice infected with *E. coli* (A; *P* = not significant) and *P. aeruginosa* (B; *P* < 0.001). . . . , control group; ———, cefoperazone-treated group; ———, cefoperazone-plus-sulbactam-treated group.

(i) **Granulocytopenia.** The mice became granulocytopenic after the second dose of cyclophosphamide. The total duration of granulocytopenia was 5 days in all three groups. Organisms were inoculated into mice on the second day of granulocytopenia.

(ii) **Serum antibiotic levels.** In experiments with *E. coli*, the mean peak cefoperazone concentrations were 274 μg/ml for the single-drug group and 247 μg/ml for the combination-drug group. For the latter group, the mean peak sulbactam level was 125 μg/ml. In experiments with *P. aeruginosa*, the mean peak cefoperazone concentrations were 255 and 236 μg/ml for the single- and combination-drug groups, respectively, and the mean peak sulbactam level for the latter group was 168 μg/ml.

(iii) **Survivors.** Figure 1 shows the survival curves for mice in experiments with *E. coli* and *P. aeruginosa*. Significant differences in survival at 48 and 72 h of therapy were noted among the three groups in the experiment with *P. aeruginosa* (*P* < 0.001). At 72 h of therapy, none of the control mice were alive, while 10 and 50% of the cefoperazone-treated and cefoperazone-plus-sulbactam-treated mice, re-
TABLE 1. Log transforms of median number of CFU per milliliter of blood and per gram of liver and spleen from mice infected with E. coli or P. aeruginosa in the control group, cefoperazone-treated group, and cefoperazone-plus-sulbactam-treated group

<table>
<thead>
<tr>
<th>Organism and sample</th>
<th>CFU for the following group:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>3.2 (2.5-3.6)</td>
</tr>
<tr>
<td>Liver</td>
<td>2.4 (2.2-2.7)</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.3 (3.9-4.8)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>5.8 (4.5-6.6)</td>
</tr>
<tr>
<td>Liver</td>
<td>7.0 (6.4-7.6)</td>
</tr>
<tr>
<td>Spleen</td>
<td>8.2 (7.7-9.1)</td>
</tr>
</tbody>
</table>

* Sample sizes for the control group, cefoperazone-treated group, and cefoperazone-plus-sulbactam-treated group were 12, 15, and 15 mice in the E. coli experiment and 16, 20, and 20 mice in the P. aeruginosa experiment, respectively. Data in parentheses represent the interquartile ranges. P for all comparisons was <0.0001.

respectively, were alive. With E. coli, no significant difference in survival was noted among the groups; however, an improved trend in survival was observed for drug-treated mice. The survival rates were 73% for the cefoperazone-plus-sulbactam-treated group, 46% for the cefoperazone-treated group, and 25% for controls.

(iv) Quantitative cultures. Table 1 shows colony counts for E. coli and P. aeruginosa in the blood, liver, and spleen of control and drug-treated mice. Despite a smaller inoculum of P. aeruginosa, larger numbers of P. aeruginosa than of E. coli were found in the blood, liver, and spleen. All three sites showed markedly smaller numbers of organisms in drug-treated mice than in control mice. Highly significant differences among the three groups (control mice and cefoperazone- and cefoperazone-plus-sulbactam-treated mice) were noted (P < 0.0001) in experiments with E. coli and P. aeruginosa. Pairwise colony count comparisons in the experiment with E. coli were all highly significant (P < 0.0001), except for colony count comparisons for the blood, liver, and spleen between control mice and cefoperazone-treated mice. In the experiment with P. aeruginosa, the difference in colony counts in the blood between control mice and cefoperazone-treated mice was not significant. However, colony counts in the liver and spleen of control versus cefoperazone-treated mice were significantly different (liver, P < 0.05; spleen, P < 0.02). All other pairwise comparisons for the P. aeruginosa experiment were highly significant (P < 0.005).

DISCUSSION

Using a granulocytopenic murine model, we clearly demonstrated in the present study that the addition of sulbactam to cefoperazone improves the efficacy of cefoperazone against infection due to cefoperazone-resistant bacteria. The previously noted in vitro superiority of the two-drug combination (3) was confirmed in this animal study. Infected mice treated with cefoperazone plus sulbactam showed improved survival as well as fewer microorganisms in the blood and in the organs examined at autopsy. Serum antibiotic levels achieved in this experiment were comparable to those achieved clinically in humans.

The primary reason for the enhanced action of cefoperazone plus sulbactam appears to be β-lactamase inhibition by sulbactam. The latter drug is very active against Richmond-Sykes class I (chromosome-mediated), III (plasmid-mediated), and V (PSE and OXA) β-lactamases (12). In addition, sulbactam in combination with penicillins has been shown to be effective against bacteria that do not produce β-lactamases (12, 17). For explanation of such activity, secondary mechanisms of action of sulbactam have been suggested. These include its affinity for binding to penicillin-binding protein 1A in cell walls of bacteria and its augmenting effect on the oxidative mechanism of polymorphonuclear leukocytes (11, 16). The former action produces morphologic changes in bacteria, making the organisms more readily susceptible to phagocytosis, and the latter action may result in an increased bactericidal capacity of polymorphonuclear leukocytes. Such modes of action of sulbactam may explain the improved activity of cefoperazone plus sulbactam against E. coli and P. aeruginosa observed in this study.

Our animal model is similar to models described by others (4, 5). Unlike the previous investigators, we inoculated the microorganisms intraperitoneally to produce disseminated infection. Although a comparatively smaller inoculum of P. aeruginosa was used to produce infection, larger numbers of P. aeruginosa than of E. coli were consistently isolated at all sites examined, suggesting a higher level of virulence of pseudomonas in the neutropenic setting.

The advantages of the combination of cefoperazone plus sulbactam over cefoperazone alone include a prolonged half-life, a prolonged postantibiotic effect, and a broadened spectrum of activity against microorganisms, including gram-negative bacilli, gram-positive cocci, and anaerobes (3, 10). The combination of cefoperazone plus sulbactam has been shown to be clinically effective in the treatment of infections in immunocompetent hosts as well as those with concomitant hematologic malignancies (7, 9). In an open, noncomparative trial, the drug combination was noted to be effective in profoundly granulocytopenic patients with serious infections, including sepsis and pneumonia (7). An response rate of 44% was seen for infected patients with persistent profound granulocytopenia. At present, there are data to support the use of cefoperazone, ceftazidime, and imipenem as monotherapeutic agents in the treatment of febrile granulocytopenic patients (14, 15, 19). Encouraging preliminary results are available from three comparative studies examining the efficacy of cefoperazone plus sulbactam in the treatment of infected, neutropenic cancer patients (1, 6, 18). With the present information, the combination of cefoperazone plus sulbactam should be considered for addition to the list of agents available for use during chemotherapy-induced granulocytopenia. This combination, if proven useful, may be reserved for the treatment of patients with known or suspected resistant gram-negative bacterial infections.

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REFERENCES


