Efficacies of Piperacillin-Tazobactam and Cefepime in Rats with Experimental Intra-Abdominal Abscesses Due to an Extended-Spectrum β-Lactamase-Producing Strain of Klebsiella pneumoniae

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The in vivo activities of piperacillin-tazobactam and cefepime were compared with those of ticarcillin-clavulanate, ceftazidime, cefotaxime, and imipenem in a rat model of intra-abdominal abscess with a strain of Klebsiella pneumoniae elaborating an extended-spectrum β-lactamase (TEM-26). With the exception of ceftazidime, all of the antimicrobial agents significantly reduced bacterial counts within abscesses at the end of therapy compared with those in untreated controls. Residual viable cell counts (mean ± standard deviation in log$_{10}$ CFU/gram) were as follows: control, 8.76 ± 0.97; ceftazidime, 8.00 ± 0.76; piperacillin-tazobactam, 3.87 ± 1.72; ticarcillin-clavulanate, 3.74 ± 1.34; cefepime, 3.15 ± 1.19; cefotaxime, 2.61 ± 0.77; imipenem, 2.41 ± 0.93. Imipenem was more effective than either of the inhibitor combinations (P < 0.05). Cefotaxime was unexpectedly effective given its poor in vivo activity against this organism in our earlier studies, which used a different dose and total duration of therapy (L. B. Rice, J. D. C. Yao, K. Klimm, G. M. Eliopoulos, and R. C. Moellering, Jr., Antimicrob. Agents Chemother. 35:1243–1244, 1991). These observations suggest that the effectiveness of cephalosporins in the treatment of experimental infections caused by extended-spectrum β-lactamase-producing K. pneumoniae may be highly dependent on dosing regimen, even for a specific organism and site of infection.

Outbreaks of infection with Enterobacteriaceae producing extended broad-spectrum β-lactamases (ESBLs) capable of hydrolyzing expanded-spectrum cephalosporins were reported from Europe in the mid-1980s (12, 29). Since that time, increasing rates of isolation of Enterobacteriaceae β-lactam antibiotics on the basis of ESBLs have been recognized as a significant problem in the United States as well (11, 14, 16, 22, 25, 27).

Because currently available β-lactamase inhibitors are active against the more commonly encountered extended-spectrum enzymes (9, 18, 20) in addition to a number of the previously recognized plasmid-mediated β-lactamases (8), their potential use in treatment of infections caused by such organisms is of interest. For example, combinations of piperacillin with tazobactam (13, 15, 23) or of cefoperazone (26), ampicillin (23), or ceftriaxone (5) with sulbactam (6) demonstrated activity in experimental animal models of infection with ESBL-producing K. pneumoniae, although in some of these studies higher than expected inhibitor–β-lactam ratios were required to demonstrate an effect. Standard tests of in vitro susceptibility to expanded-spectrum cephalosporins of strains bearing ESBLs are not always predictive of activity in vivo. In our previous studies of an intra-abdominal abscess model with K. pneumoniae 5657 (TEM-26), cefoperazone, cefotaxime, and cefpirome were inactive when administered by continuous infusion to achieve concentrations in serum well above the MICs (1 to 2 μg/ml) determined with standard inocula (26). In a model of Escherichia coli (SHV-2) endocarditis, ceftriaxone alone was ineffective despite activity in vitro; cures were obtained with the addition of sulbactam (6). Activities of these agents in vitro were markedly affected adversely by increases in the size of the bacterial inoculum, and it is likely that conditions of the abscess milieu (limited antibiotic penetration, a large bacterial inoculum) favor local antibiotic inactivation.

This study was undertaken to compare the activities of piperacillin-tazobactam and cefepime with those of ticarcillin-clavulanate and imipenem, each given by intermittent intravenous infusion, in the intra-abdominal abscess model with K. pneumoniae 5657 (TEM-26) (26). Previous work by Rice et al. (23) and in our laboratory (26) with this model has demonstrated that piperacillin-tazobactam (8:1) administered by continuous intravenous infusion was effective in reducing bacterial density within abscesses, but less so than imipenem, while cefpirome was inactive in vivo despite activity in vitro (MIC, 1.0 μg/ml) against the test strain. In the present study, antibiotics were administered in intermittent intravenous (i.v.) doses at intervals intended to achieve peak levels in serum comparable to those achieved in humans, with trough levels in serum not to exceed those expected in clinical practice (2–4, 33, 34).

We included as controls ceftazidime, which was inactive in vitro (MIC, 128 μg/ml), and cefotaxime, an agent which was active in vitro (MIC, 1.0 μg/ml) but shown to be inactive in vivo in our previous work (26). Surprisingly, the latter agent proved effective under the conditions of this study.

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TABLE 1. In vitro susceptibility of K. pneumoniae 5657 (TEM-26) to study antibiotics

<table>
<thead>
<tr>
<th>Antimicrobial agent(s)</th>
<th>MIC (μg/ml) of antibiotic for inoculum concn of:</th>
<th>10^3 CFU/ml</th>
<th>10^4 CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin</td>
<td>128</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam (8:1)</td>
<td>8</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td></td>
</tr>
<tr>
<td>Ticarcillin-clavulanate</td>
<td>32</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1</td>
<td>&gt;64</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>128</td>
<td>&gt;512</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>=0.06</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

a MICs are in micrograms of piperacillin per milliliter.
b MICs are in micrograms of ticarcillin per milliliter; clavulanate was used at a fixed concentration of 2 μg/ml.

MATERIALS AND METHODS

Test organism. The infecting strain used in this study, K. pneumoniae 5657, was a clinical isolate from sputum producing a TEM-26 β-lactamase as previously described (24–26).

Testing of susceptibility to antimicrobial agents. The susceptibility of the test strain to the antibiotics used in this study was determined in cation-adjusted Mueller-Hinton II broth (BBL, Cockeysville, Md.) by a microdilution technique used in accordance with National Committee for Clinical Laboratory Standards guidelines (17). Testing was performed by using inoculum concentrations of approximately 10^3 and 10^4 CFU/ml.

Antimicrobial agents. Piperacillin-tazobactam was a gift from American Cyanamid Co., Medical Research Division, Pearl River, N.Y.; ceftazidime was from Hoechst Marion Roussel Inc., Kansas City, Mo.; and imipenem-clavistatin was from Merck & Co., Inc., West Point, Pa. Cefepime was provided by SmithKline Beecham Pharmaceuticals, Philadelphia, Pa.

Inoculum preparation. The infecting strain of K. pneumoniae was grown over night in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and then diluted (1:10,000) with the same broth to reach a colony count of approximately 10^5 CFU/ml. This was mixed with heat-killed Bacteroides fragilis and sterilized, pooled rat fecal contents in a ratio of 1:1:2.

Abscess formation. Intra-abdominal abscesses were created in male Sprague-Dawley rats (Taconic, Germantown, N.Y.) weighing ca. 200 g. Following anesthesia with ketamine and xylazine, a 1.5-cm midline abdominal incision was made and a 0.0 gelatin capsule containing 0.5 ml of the inoculum mixture (approximately 7.5 × 10^5 CFU of K. pneumoniae) was implanted in the peritoneal cavity. Deep muscle layers were closed with no. 3 silk sutures, and the skin was closed with metal surgical clips.

Antibiotic therapy. Antimicrobial agents were administered via a surgically implanted central venous catheter inserted through the left jugular vein into the apex of the right ventricle. Deep muscle layers were closed with no. 3 silk sutures, and the skin was closed with metal surgical clips.

The susceptibility of the test organism, K. pneumoniae ATCC 25922, was determined by using a E. coli strain as previously described (9). The test organisms were E. coli ATCC 25922 for cefepime and B. subtilis spores (Difco Laboratories) for piperacillin, ticarcillin, and imipenem. Since high-performance liquid chromatography was not available for measurement of ceftazidime levels in serum, the agar well diffusion bioassay method was used with B. subtilis spores as the test organism. This technique measured the total active drug (the parent compound plus the desacetyl metabolite) and gave results in good agreement with those obtained in our previous studies in which high-performance liquid chromatography was available to measure ceftaxime and desacetyl ceftaxime levels separately (19, 26). At 4.5 days, animals were sacrificed approximately 2 to 3 h after the last dose or the end of the continuous i.v. infusion. Abscess contents were removed aseptically, weighed, and suspended in 2 ml of sterile saline. These samples were homogenized, serially diluted, and plated on blood agar and MacConkey (BBL) plates in duplicate. After 24 to 48 h of incubation, results were read. Considering the heterogeneity in the weights of the abscess contents, the limits of detection by our method ranged from 1.02 to 1.74 log_10 CFU/g. When no bacterial growth was noted, the value of the limit of detection for the specific animal was entered in the statistical analysis.

Statistical solution. The Fischer exact test was used to evaluate nominal data such as abscess content stability. Statistical analysis for differences in abscess bacterial densities for the various regimens employed analysis of variance followed by t tests using Bonferroni’s correction for multiple comparisons.

RESULTS

Susceptibility studies for the infecting strain. Table 1 shows the susceptibility of K. pneumoniae 5657 to the antimicrobial agents used in this study. As expected, addition of a β-lactamase inhibitor to either piperacillin or ticarcillin substantially decreased the MICs of these penicillins. The activities of all cephalosporins were subject to a marked inoculum effect. Even though the MIC of imipenem at an inoculum concentration of 10^7 CFU/ml was at least four times as high as that observed with 10^5 CFU/ml, this antibiotic was the most active in vitro against the infecting strain with either inoculum concentration.

Antibiotic levels in serum. Levels of antibiotics attained in the sera of animals are shown in Table 2. The mean peak levels of all antimicrobial agents determined 5 min after the end of the i.v. infusion were comparable to levels achievable in humans with parenteral therapy and exceeded the MICs of the antibiotics for the infecting strain, except for ceftazidime, which was administered by continuous i.v. infusion. Levels of ceftaxime were comparable to those attained with this dose previously (19). For the additional regimens tested with cefo-

TABLE 2. Outcome of therapy for intra-abdominal abscesses due to K. pneumoniae 5657

<table>
<thead>
<tr>
<th>Antimicrobial agent(s)</th>
<th>Dosage (mg/kg), frequency</th>
<th>No. of animals</th>
<th>Mean antibiotic level in serum ≥ SD (μg/ml)</th>
<th>Mean bacterial density in abscess ≥ SD (log_{10} CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak</td>
<td>Trough</td>
</tr>
<tr>
<td>No treatment</td>
<td></td>
<td>31</td>
<td>8.76 ± 0.97</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>400, q24h</td>
<td>10</td>
<td>19.4 ± 3.09a</td>
<td>8.00 ± 0.76</td>
</tr>
<tr>
<td>Piperacillin + tazobactam</td>
<td>225, q2h</td>
<td>14</td>
<td>317 ± 16.6</td>
<td>&lt;3.12</td>
</tr>
<tr>
<td>Ticarcillin + clavulanate</td>
<td>225, q2h</td>
<td>14</td>
<td>258 ± 97.9</td>
<td>≥6.02</td>
</tr>
<tr>
<td>Cefepime</td>
<td>60, q3h</td>
<td>17</td>
<td>109 ± 48</td>
<td>1.79 ± 0.34</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>180, q4h</td>
<td>21</td>
<td>293 ± 40.4</td>
<td>3.12</td>
</tr>
<tr>
<td>Imipenem</td>
<td>40, q2.5h</td>
<td>15</td>
<td>93 ± 30</td>
<td>≤0.78</td>
</tr>
</tbody>
</table>

a Ceftazidime was administered by continuous i.v. infusion; the level in serum reported here was obtained in a previous study with the same dosage (26).
b The dosage and the levels in serum obtained with the regimen shown are those of piperacillin.

The dosage and the levels in serum obtained with the regimen shown are those of ticarcillin.
taxime, levels of the total drug in serum were as follows. With the same daily dose of cefotaxime as in Table 2 but given by continuous i.v. infusion (i.e., 1,000 mg/kg/24 h), the mean ± standard deviation (SD) level in serum was 108 ± 10.4 µg/ml. With the lower dose of cefotaxime (400 mg/kg/24 h), levels in serum were 32.33 ± 2.52 µg/ml for the continuous i.v. infusion. Peak and trough levels were 153 ± 20.2 and <3.12 µg/ml, respectively, for the intermittent i.v. infusion at this lower dose (i.e., 67 mg/kg q4h).

**Experimental intra-abdominal abscesses.** Bacterial densities in the abscess contents after treatment are shown in Table 2. With the exception of cefotaxime, all treatment groups had significantly reduced bacterial densities in abscesses compared with untreated controls (P < 0.05). No animal had sterile abscess contents in either the untreated control group or the cefotaxide group. Both the piperacillin-tazobactam and ticarcillin-clavulanate regimens eradicated the infecting strain from abscesses in only one animal each in group. The imipenem, ceftazidime, and cefotaxime regimens each reduced organisms in abscess contents to below the limits of detection in samples from five animals each from group; this was statistically significantly different from controls (P < 0.05). Imipenem was more effective in decreasing colony counts in abscess contents than either piperacillin-tazobactam or ticarcillin-clavulanate (P < 0.05). Cefotaxime was also more effective than piperacillin-tazobactam (P < 0.05). Residual bacterial densities in the imipenem, ceftazidime, and cefotaxime treatment groups were not significantly different.

In a previous study done in our laboratory by using the same model of intra-abdominal abscesses due to the same infecting strain (26), cefotaxime given by continuous i.v. infusion at a lower daily dose (i.e., 400 mg/kg/24 h) was ineffective compared with untreated controls (the residual bacterial densities were 7.26 ± 1.02 and 8.02 ± 1.02 CFU/g, respectively). To determine whether the unexpectedly good results observed in the present study resulted from differences in the total daily dose or the modes of administration between the two studies, we reevaluated the efficacy of cefotaxime at the previously used lower daily dose given either by continuous i.v. infusion (i.e., 400 mg/kg/24 h) or by intermittent i.v. infusion (i.e., 67 mg/kg q4h) and also at the higher dose used in the present study delivered by continuous i.v. infusion (i.e., 1,000 mg/kg/24 h).

Results are shown in Table 3. Although the 400-mg/kg/24 h continuous-infusion regimen reduced bacterial counts significantly compared with untreated controls (P < 0.05), this regimen was significantly less effective than both the intermittent regimen with the same daily dose and the continuous-infusion regimen with the higher dose of 1,000 mg/kg/24 h. Studies with the 1,000-mg/kg/24 h dose were done in two parts; by coincidence, the inocula for these differed by 1 log_{10} CFU. There was a trend toward a modest reduction in effectiveness of cefotaxime against the higher-inoculum group (2.91 ± 0.74 log_{10} CFU/g versus 2.27 ± 0.69 log_{10} CFU/g), but this difference did not achieve statistical significance (P = 0.055).

**DISCUSSION**

Piperacillin-tazobactam (8:1) was found to be effective in this model when given in doses that resulted in peak piperacillin concentrations in serum within the range observed in humans after a 4-g i.v. dose (33, 34) and which yielded trough concentrations in serum below the limits of detection. Results obtained with this combination were virtually identical to those obtained with ticarcillin-clavulanate, even though the latter was fourfold less active in vitro with both inocula. The final bacterial density in abscesses which was determined after treatment of animals with piperacillin-tazobactam (3.87 ± 1.72 log_{10} CFU/g) was 10-fold lower than that obtained by Rice et al. (23) (4.97 ± 1.48 log_{10} CFU/g), which may be due to the 50% longer duration of therapy (4.5 days versus 3 days), to the approximately 75% higher daily dose (2,700 mg versus 1,500 mg), or to the high peak concentrations obtained by intermittent infusions in the present study. Imipenem was significantly more active than piperacillin-tazobactam here and in the study of Rice et al. (23).

Among 108 isolates of *E. coli* and *Klebsiella* spp. identified as putative ESBL producers by Jett et al. (10), 88% were susceptible to piperacillin-tazobactam. Those investigators, however, called attention to the fact that in contrast to the relatively low MICs of imipenem, piperacillin-tazobactam inhibited most isolates only at concentrations within 1 or 2 dilutions of the susceptibility breakpoint concentration and was subject to substantial inoculum effects on MICs and MBCs, suggesting that caution should be exercised in using this combination for treatment of serious infections due to ESBL-producing organisms. Although TEM-26-producing isolates of *K. pneumoniae* such as the strain used in our study have been recovered from outbreaks in Massachusetts, New York, and California (16, 24, 25), a wide variety of other ESBLs have been encountered (9, 20). We further caution that results different from ours might be obtained with *K. pneumoniae* producing other ESBLs. In animal models using other ESBL-producing strains, higher ratios of tazobactam to piperacillin (i.e., 1:4) were superior to the standard 1:8 ratio available for clinical use (7, 13). Furthermore, the activity of a β-lactam against ESBL-producing *Enterobacteriaceae* would depend not only on the specific ESBL also but also on the quantity of enzyme produced and on the relative impermeability of the bacterial outer membrane to the β-lactam which might arise from porin channel alterations.

The fact that ceftazidime, which was active in vitro against an inoculum concentration of 10^9 CFU/ml but not against 10^7 CFU/ml, was as active as imipenem in this model could not have been altogether anticipated. This occurred with peak levels in serum just under those achieved with 2-g i.v. doses in

<table>
<thead>
<tr>
<th>Daily dose (mg/kg)</th>
<th>Regimen</th>
<th>No. of animals (inoculum size)*</th>
<th>Mean peak/trough levels or mean level in serum (µg/ml) ± SD</th>
<th>Mean abscess bacterial density ± SD (log_{10} CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>Continuous infusion</td>
<td>10 (10^6 CFU)</td>
<td>293 ± 40.4/3.12</td>
<td>2.27 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td></td>
<td>11 (10^6 CFU)</td>
<td>2.91 ± 0.74</td>
</tr>
<tr>
<td>1,000</td>
<td>Continuous infusion</td>
<td>12</td>
<td>108 ± 10.4</td>
<td>2.87 ± 1.01</td>
</tr>
<tr>
<td>400</td>
<td>Continuous infusion</td>
<td>12</td>
<td>153 ± 20.2/3.12</td>
<td>2.73 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>Continuous infusion</td>
<td>9</td>
<td>32.33 ± 2.52</td>
<td>4.28 ± 0.64</td>
</tr>
</tbody>
</table>

* Inocula were approximately 10^6 CFU unless indicated otherwise.
ACKNOWLEDGMENTS

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REFERENCES


