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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 regimens, 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 *Klebsiella pneumoniae* and other *Enterobacteriaceae* resistant to ceftazidime and various other 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 have shown 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 μ/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

Antimicrobial agent(s)	MIC (μg/ml) of antibiotic for inoculum concn of:	
	10^5 CFU/ml	10^7 CFU/ml
Piperacillin	128	>256
Piperacillin-tazobactam (8:1)[a]	8	16
Ticarcillin	>512	>512
Ticarcillin-clavulanate[b]	32	64
Cefepime	1	>64
Cefotaxime	0.25	64
Ceftazidime	128	>512
Imipenem	≤0.06	0.25

[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 10^5 and 10^7 CFU/ml.

Antimicrobial agents. Piperacillin-tazobactam was a gift from American Cyanamid Co., Medical Research Division, Pearl River, N.Y.; cefotaxime was from Hoechst Marion Roussel Inc., Kansas City, Mo.; and imipenem-cilastatin was from Merck & Co., Inc., West Point, Pa. Cefepime was provided by Bristol-Myers Squibb, Princeton, N.J., and ceftazidime was from Eli Lilly & Co., Indianapolis, Ind. Ticarcillin-clavulanate was purchased from SmithKline Beecham Pharmaceuticals, Philadelphia, Pa.

Inoculum preparation. The infecting strain of *K. pneumoniae* was grown overnight 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 6×10^5 CFU/ml. This was mixed with heat-killed *Bacteroides fragilis* and sterilized, pooled rat cecal 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 no. 00 gelatin capsule containing 0.5 ml of the inoculum mixture (approximately 7.5×10^4 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 superior vena cava as previously described (31). Therapy was started 4 h after capsule implantation and continued for 4.5 days. Antibiotic doses and intervals between infusions were selected for each antimicrobial agent to reach clinically relevant levels. Animals were randomly assigned to one of the following seven groups: (i) piperacillin (225 mg/kg of body weight every 2 h [q2h]) plus tazobactam in an 8:1 ratio, (ii) ticarcillin (225 mg/kg q2h) plus clavulanate in a ratio of 30:1, (iii) cefepime (60 mg/kg q3h), (iv) cefotaxime (180 mg/kg q4h), (v) imipenem-cilastatin (40 mg/kg q2.5h), (vi) ceftazidime (400 mg/kg/24 h administered by continuous i.v. infusion), and (vii) a negative control (untreated animals). Delivery of intermittent antibiotic infusions in accordance with the schedules above was accomplished by use of a syringe pump (Harvard) controlled by an electronic timer. In view of the unexpectedly good results obtained with cefotaxime (180 mg/kg q4h), additional studies were performed with this antimicrobial agent by using the same daily dose but delivering it by continuous i.v. infusion (1,000 mg/kg/24 h) or by using the lower dosage used in our previous study (26) given either by continuous i.v. infusion (400 mg/kg/24 h) or intermittently (67 mg/kg q4h).

Monitoring of therapy and outcome. On day 3 or 4 of therapy, antibiotic levels in serum were measured by agar well diffusion bioassay (1). 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 cefotaxime 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 cefotaxime and desacetyl cefotaxime 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 resuspended 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 sterility. 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 cefotaxime 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

Antimicrobial agent(s)	Dosage (mg/kg), frequency	No. of animals	Mean antibiotic level in serum ± SD (μg/ml)		Mean bacterial density in abscess ± SD ($\log_{10}$ CFU/g)
			Peak	Trough	
No treatment		31			8.76 ± 0.97
Ceftazidime	400, q24h	10	19.4 ± 3.09[a]		8.00 ± 0.76
Piperacillin[b] + tazobactam	225, q2h	14	317 ± 16.6	<3.12	3.87 ± 1.72
Ticarcillin[c] + clavulanate	225, q2h	14	258 ± 97.9	≤6.02	3.74 ± 1.34
Cefepime	60, q3h	17	109 ± 48	1.79 ± 0.34	3.15 ± 1.19
Cefotaxime	180, q4h	21	293 ± 40.4	3.12	2.61 ± 0.77
Imipenem	40, q2.5h	15	93 ± 30	≤0.78	2.41 ± 0.93

[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.
[c] The dosage and the levels in serum obtained with the regimen shown are those of ticarcillin.

TABLE 3. Effect of dosing regimen on activity of cefotaxime against *K. pneumoniae* (TEM-26) in experimental intra-abdominal abscess

Daily dose (mg/kg)	Regimen	No. of animals (inoculum size)[a]	Mean peak/trough levels or mean level in serum (μg/ml) ± SD	Mean abscess bacterial density ± SD ($\log_{10}$ CFU/g)
1,000	180 mg/kg q4h	10 (10^5CFU)	293 ± 40.4/<3.12	2.27 ± 0.69
		11 (10^6CFU)		2.91 ± 0.74
1,000	Continuous infusion	12	108 ± 10.4	2.87 ± 1.01
400	67 mg q4h	11	153 ± 20.2/<3.12	2.73 ± 0.86
400	Continuous infusion	9	32.33 ± 2.52	4.28 ± 0.64

[a] Inocula were approximately 10^5 CFU unless indicated otherwise.

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 ceftazidime, 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 ceftazidime group. Both the piperacillin-tazobactam and ticarcillin-clavulanate regimens eradicated the infecting strain from abscesses in only one animal in each group. The imipenem, cefepime, and cefotaxime regimens each reduced organisms in abscess contents to below the limits of detection in samples from five animals from each 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, cefepime, 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 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 cefepime, which was active in vitro against an inoculum concentration of 10^5 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

human volunteers (levels of approximately 130 μg/ml) and with trough concentrations at or below those expected in clinical use (3, 4). Although cefepime is more active than several other cephalosporins against β-lactamase (including ESBL)-producing *Enterobacteriaceae* (28), Jett et al. (10) reported that the drug inhibited only 52% of putative ESBL-producing *Klebsiella* spp., again subject to a marked inoculum effect.

As expected, ceftazidime was ineffective in this model. However, the effectiveness of cefotaxime was surprising in view of its lack of effect in our previous study, in which the antibiotic was administered by continuous infusion at a dose of 400 mg/kg/day for 3 days (26). Of the four dosing regimens which we studied to investigate explanations for this observation (Table 3), it was clear that this dose (which resulted in mean concentrations in serum virtually identical to those achieved in our previous work) was the least effective. The lower residual bacterial density observed with this dose in the present study (4.28 ± 0.64 $\log_{10}$ CFU/g) compared with the previous experiments (7.26 ± 1.02 $\log_{10}$ CFU/g [26]) may have resulted from the longer duration of therapy used here.

There is limited information indicating that cefotaxime or other cephalosporins have been used successfully in some patients with infections due to ceftazidime-resistant *K. pneumoniae* (21, 25, 30); however, failures have also been noted (16). The results of our two studies with cefotaxime in this model, while initially appearing contradictory, actually help to explain these clinical observations. They suggest that a successful outcome may be highly dependent on the dosing regimens used and the resulting drug concentrations in serum attained, even for a specific ESBL-producing strain and site of infection. If the outcome is so critically dependent on the dosing regimens employed, it may be wise to avoid routine use of expanded-spectrum cephalosporins against infections caused by EBSL-producing strains until factors influencing the likelihood of their successful application are better understood.

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