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In Vivo Efficacies of β-Lactam–β-Lactamase Inhibitor Combinations against a TEM-26-Producing Strain of *Klebsiella pneumoniae*

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We examined the efficacies of the β-lactam–β-lactamase inhibitor combinations ampicillin-sulbactam and piperacillin-tazobactam in the treatment of intra-abdominal abscesses caused by a TEM-26-producing strain of *Klebsiella pneumoniae*. At lower doses, both combinations reduced abscess colony counts by more than 3 $\log_{10}$ CFU/g from that of untreated controls, but treatment with these drugs was inferior to treatment with imipenem. Increasing the doses of the combinations resulted in a further decrease in abscess CFU to a level where both were similar to imipenem in efficacy. These results suggest that the β-lactam–β-lactamase inhibitor combinations ampicillin-sulbactam and piperacillin-tazobactam may be viable alternatives for the treatment of serious infections caused by susceptible extended-spectrum β-lactamase-producing strains of *K. pneumoniae*.

Strains of *Klebsiella pneumoniae* resistant to extended-spectrum cephalosporins by virtue of the production of extended-spectrum β-lactamases are becoming increasingly common in hospitals around this country (3). Although strains producing these enzymes demonstrate a fairly wide range of MICs to different extended-spectrum cephalosporins, the majority are highly resistant to all cephalosporins (with the exception of cephamycins such as cefoxitin and cefotetan) when tested in high inocula. Results of animal model experiments suggest that extended-spectrum β-lactamase-producing organisms will be resistant to all extended-spectrum cephalosporins, regardless of the standard inoculum MICs (9). In support of this conclusion, therapeutic failures of extended-spectrum cephalosporins in the treatment of infections caused by ceftazidimase-producing *K. pneumoniae* have been reported (4).

Most extended-spectrum β-lactamases are derived from the TEM-type β-lactamases, the most common of the plasmid-encoded enzymes found in the family *Enterobacteriaciae*. TEM-related extended-spectrum enzymes generally retain susceptibility to β-lactamase inhibitors, suggesting that the use of β-lactam–β-lactamase inhibitor combinations will be a viable strategy for the treatment of infections caused by these strains (3). Relatively little in vivo data exists, however, supporting the efficacies of such combinations in the treatment of clinically relevant infections caused by extended-spectrum β-lactamase-producing strains of *K. pneumoniae* (2, 9). We report a comparison of two currently available β-lactam–β-lactamase inhibitor combinations, ampicillin-sulbactam and piperacillin-tazobactam, in the treatment of rat intra-abdominal abscesses caused by a TEM-26-producing strain of *K. pneumoniae*.

K. pneumoniae 5657 is a TEM-26 (formerly YOU-1)-producing clinical isolate shown previously to be resistant to several extended-spectrum cephalosporins in a rat intra-abdominal abscess model (7, 8). In vitro MICs were determined by using a microbroth dilution technique in Luria-Bertani medium (5) with starting inocula of 2.16×10^5 and 2.1×10^7 CFU/ml. Rat intra-abdominal abscesses were induced as previously described (6, 9). In brief, gelatin capsules containing a mixture of sterilized rat cecal contents, killed *Bacteroides fragilis* 25285 (encapsulated strain), and *K. pneumoniae* 5657 (10^5 CFU) in a 2:1:1 ratio were surgically implanted into the peritoneums of 150- to 200-g male Sprague-Dawley rats. Therapy was begun via continuous intravenous infusion into the jugular vein 2 to 3 h after capsule implantation. Ampicillin-sulbactam was administered in two different doses (either 500 mg of ampicillin per kg of body weight per day and 250 mg of sulbactam per kg per day or 1,000 mg of ampicillin per kg per day and 500 mg of sulbactam per kg per day). Imipenem was administered in a dose of 300 mg/kg/day. Piperacillin-tazobactam was administered in two different doses (either 750 mg of piperacillin per kg per day and 100 mg of tazobactam per kg per day or 1,500 mg of piperacillin per kg per day and 200 mg of tazobactam per kg per day). Therapy was continued for 3 days, after which the rats were euthanized and the abscesses were excised in a sterile fashion, weighed, and diluted for colony counting with Luria-Bertani agar plates containing ceftazidime (5 μg/ml). Results were expressed as CFU per gram of abscess. Serum was sampled after 24 h for determination of antimicrobial concentrations. Ampicillin, imipenem, and piperacillin concentrations were measured by a bioassay with *Bacillus subtilis* spores (ATCC 6633) (1). Because of technical problems, sulbactam and tazobactam concentrations in serum were not measured. Prior experiments suggest that serum sulbactam concentrations are roughly 50 to 75% of the serum ampicillin concentrations (6). Results for each group were compared by analysis of variance followed by Student's *t* test for comparing independent variables. By using Bonferroni's correction for comparing multiple variables, a *P* value of <0.01 was set for assigning statistical significance.

K. pneumoniae 5657 MICs (in micrograms per milliliter) to various antimicrobial agents were as follows: ampicillin, >256; sulbactam, 32; ampicillin-sulbactam (2:1), 8; ceftazidime, 256; imipenem, 0.5; piperacillin, >256; tazobactam, 256; and piperacillin-tazobactam (8:1), 8. Increasing the inoculum 100-fold (10^7 CFU/ml) resulted in a two- to five-doubling dilution increase in the MICs for the antimicrobial combinations and imipenem (ampicillin-sulbactam, 32 μg/ml; piperacillin-tazobactam, 64 μg/ml; imipenem, 16 μg/ml). Increasing the inoculum 100-fold did not alter the MICs of the inhibitors alone.

Results of the experiments are shown in Table 1. Overall,

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TABLE 1. Results of abscess treatment with ampicillin-sulbactam, piperacillin-tazobactam, and imipenem[a]

Antibiotic(s)	Dose (mg/kg/day)	No. of rats	Mean serum concentration (μg/ml) (± SD)	Log_{10} CFU/g of abscess (± SD)
None		22		9.5 ± 0.53
Ampicillin-sulbactam A	500,250	12	29 ± 10.7	5.91 ± 0.85
Piperacillin-tazobactam A	750,100	5	24.5 ± 2.1	6.2 ± 1.6
Ampicillin-sulbactam B	1,000,500	6	38 ± 4.2	4.38 ± 0.32
Piperacillin-tazobactam B	1,500,200	15	32.3 ± 8.5	4.97 ± 1.48
Imipenem	300	8	11.1 ± 3.1	3.76 ± 0.91

[a] All treatment groups versus controls, $P < 0.000$ (statistically significant); ampicillin-sulbactam B versus imipenem, $P = 0.14$; piperacillin-tazobactam B versus imipenem, $P = 0.048$; ampicillin-sulbactam B versus piperacillin-tazobactam B, $P = 0.35$; ampicillin-sulbactam A versus imipenem, $P < 0.000$ (statistically significant); piperacillin-tazobactam A versus imipenem, $P < 0.004$ (statistically significant); ampicillin-sulbactam A versus piperacillin-tazobactam A, $P = 0.65$; ampicillin-sulbactam A versus piperacillin-tazobactam B, $P = 0.063$.

imipenem was the most effective antibiotic tested, resulting in an approximately 6 log_{10} CFU/g decrease from that of untreated controls. At the lower doses, both ampicillin-sulbactam and piperacillin-tazobactam demonstrated a decrease of greater than 3 log_{10} CFU/g from that of untreated controls, but these drug combinations were inferior to imipenem. At the higher doses, both ampicillin-sulbactam and piperacillin-tazobactam reduced abscess colony counts by more than 1 log_{10} CFU/g from that of the lower doses of each combination. The results of high-dose ampicillin-sulbactam and piperacillin-tazobactam treatment were not statistically distinguishable from treatment with imipenem.

To date, imipenem has been proffered as the treatment of choice for infections caused by extended-spectrum β-lactamase-producing strains of *K. pneumoniae* because of its resistance to hydrolysis by extended-spectrum enzymes, its activity in prior animal model experiments, and a growing body of evidence that it is effective in the clinical setting (3, 4, 9). The increasing prevalence of ceftazidime-resistant *Klebsiella* strains and the desire to restrict the use of imipenem argue for the exploration of other therapeutic alternatives. The results reported in this study suggest that the β-lactam–β-lactamase inhibitor combinations ampicillin-sulbactam and piperacillin-tazobactam may be viable alternatives for the treatment of serious infections caused by extended-spectrum β-lactamase-producing strains of *K. pneumoniae* which demonstrate in vitro susceptibility to these agents.

It is inherently problematic to use results of animal experiments to justify treatment strategies for the therapy of human infections. For the experiments reported herein, it is difficult to know the doses of β-lactam–β-lactamase inhibitor combinations which will achieve maximal effect in humans. By using the rough estimate that the mean concentration of a β-lactam antibiotic in serum in humans (with intermittent dosing) approximates one-third of the peak serum concentration, the mean serum concentrations of ampicillin and piperacillin seen in the animals used in these experiments fall well within the anticipated mean when standard doses (2 g of ampicillin [peak, ca. 120 μg/ml] and 3 g of piperacillin [peak, ca. 200 μg/ml]) of β-lactam–β-lactamase inhibitor combinations are used (3a, 5a).

Non-β-lactam antimicrobial agents such as the fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole remain viable alternatives for the treatment of extended-spectrum β-lactamase-producing *Klebsiella* strains susceptible to these agents. Unfortunately, the frequent presence of genes encoding these enzymes on large, multiresistance plasmids often results in a marked decrease in viable non-β-lactam therapeutic alternatives (3). The strain used in these experiments, for example, encodes ceftazidime resistance on a large plasmid which also encodes resistance to chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole (8). Evidence for the clinical efficacy of β-lactam–β-lactamase inhibitor combinations against these strains, as provided by this study, provides important information about the therapeutic options available for treatment of nosocomial infections caused by resistant pathogens.

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