ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1997, p. 721–727 Vol. 41, No. 4
0066-4804/97/$04.00+0

Importance of β-Lactamase Inhibitor Pharmacokinetics in the Pharmacodynamics of Inhibitor-Drug Combinations: Studies with Piperacillin-Tazobactam and Piperacillin-Sulbactam

PHILIP D. LISTER,* ANDREA M. PREVAN, AND CHRISTINE C. SANDERS

Center for Research in Anti-Infectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska 68178

Received 20 May 1996/Returned for modification 16 October 1996/Accepted 6 January 1997

An in vitro pharmacokinetic model was used to study the pharmacodynamics of piperacillin-tazobactam and piperacillin-sulbactam against gram-negative bacilli producing plasmid-encoded β-lactamases. Logarithmic-phase cultures were exposed to peak antibiotic concentrations observed in human serum after the administration of intravenous doses of 3 g of piperacillin and 0.375 g of tazobactam or 0.5 g of sulbactam. Piperacillin and inhibitor were either dosed simultaneously or piperacillin was dosed sequentially 0.5 h after dosing with the inhibitor. In studies with all four test strains, the pharmacodynamics observed after simultaneous dosing were similar to those observed with the sequential regimen. Since the ratio between piperacillin and tazobactam was in constant fluctuation after sequential dosing, these data suggest that the pharmacodynamics of the piperacillin-inhibitor combinations were not dependent upon maintenance of a critical ratio between the components. Furthermore, when regrowth was observed, the time at which bacterial counts began to increase was similar between the simultaneous and sequential dosing regimens. Since the pharmacokinetics of the inhibitors were the same for all regimens, these data suggest that the length of time that the antibacterial activity was maintained over the dosing interval with these combinations was dictated by the pharmacokinetics of the β-lactamase inhibitor in the combination. The antibacterial activity of the combination appeared to be lost when the amount of inhibitor available fell below some critical concentration. This critical concentration varied depending upon the type and amount of enzyme produced, as well as the specific inhibitor used. These results indicate that the antibacterial activity of drug-inhibitor combinations, when dosed at their currently recommended ratios, is more dependent on the pharmacokinetics of the inhibitor than on those of the β-lactam drug.

Piperacillin-tazobactam is the fourth combination of a β-lactam antibiotic and a β-lactamase inhibitor to gain U.S. Food and Drug Administration approval for clinical use. The in vitro spectrum of piperacillin-tazobactam is broader than those of its predecessors, ampicillin-sulbactam, ticarcillin-clavulanate, and amoxicillin-clavulanate, and its potency is generally higher (10, 12, 17). The relative increased activity of piperacillin-tazobactam can be partly attributed to the increased potency of piperacillin compared to that of ticarcillin and the broadened spectrum of piperacillin compared to those of the aminopenicillins (5, 7, 17). Also involved in the increased activity of piperacillin-tazobactam is the potency of tazobactam as an inhibitor of β-lactamase. Against plasmid-mediated β-lactamases the potency of tazobactam is generally comparable to that of clavulanate and is greater than that of sulbactam (3, 4, 17).

Current therapeutic practice with piperacillin-tazobactam is to dose the two components simultaneously at a ratio of 8:1 (piperacillin:tazobactam). Due to their similar pharmacokinetics in humans (18), these two components generally maintain an 8:1 ratio at the site of infection over the entire dosing interval. The pharmacodynamic principles that govern the efficacy of piperacillin-tazobactam and other β-lactamase inhibitor–β-lactam combinations remain largely unknown. It has been suggested that the maintenance of a critical ratio between the components is essential for optimum bactericidal activity. However, no data have been published to support this theory. Recent studies have suggested that the bactericidal activity of ampicillin-sulbactam against TEM-1-producing *Escherichia coli* is not dependent upon the maintenance of a 2:1 ratio between the components (1). Furthermore, those studies highlighted the importance of the sulbactam pharmacokinetics in dictating how long antibacterial activity was maintained after dosing. Therefore, a second study was designed to further assess the pharmacodynamic principles governing efficacy of inhibitor-drug combinations. In this study, the pharmacodynamics of piperacillin-tazobactam against a panel of two *E. coli* isolates producing TEM-1 and TEM-10 β-lactamases and two *Klebsiella pneumoniae* isolates producing SHV-1 and SHV-2 β-lactamases were evaluated in an in vitro pharmacokinetic model (IVPM). To evaluate the importance of maintaining a ratio of 8:1 between piperacillin and tazobactam on the antibacterial activity of the combination, the pharmacodynamics observed after simultaneous administration of both components were compared with those observed when piperacillin was administered 0.5 h after dosing with tazobactam. Finally, to compare the activities of tazobactam and sulbactam, similar studies were performed with a combination of piperacillin-sulbactam, in which the kinetics of sulbactam are the same as those of tazobactam. Data generated from these studies were used to assess the relative importance of each component to the observed antibacterial activities of the combinations, as well as to assess differences between the two inhibitors.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Four strains were evaluated in this study. These strains included two *E. coli* strains and two *K. pneumoniae* strains that expressed resistance to piperacillin through the production of plasmid-mediated β-lactamases. Resistance in the *E. coli* strains was mediated through production of either the TEM-1 (*E. coli* MISC119) or the TEM-10 (*E. coli* PABC10) β-lactamase. Resistance in the *K. pneumoniae* strains was mediated

* Corresponding author. Phone: (402) 280-1881. Fax: (402) 280-1225.

TABLE 1. Characteristics of test strains

Strain	β-Lactamase produced	β-Lactamase activity[a]	MIC (μg/ml)[b]				
			Piperacillin	Tazobactam	Sulbactam	Piperacillin-tazobactam	Piperacillin-sulbactam
E. coli MISC119	TEM-1	2,677	>512	>128	128	0.25/4	128/4
E. coli PABC10	TEM-10	396	>512	>128	64	≤0.06/4	0.25/4
K. pneumoniae GB89	SHV-1	8,005	>512	>128	>128	>256/4	>256/4
K. pneumoniae 192	SHV-2	4,671	>512	>128	>128	>256/4	>256/4

[a] Nanomoles of piperacillin hydrolyzed per minute per milligram of protein.
[b] Agar dilution susceptibility testing was performed by the procedures recommended by the National Committee for Clinical Laboratory Standards (13). For piperacillin-tazobactam and piperacillin-sulbactam, tests were performed with fixed concentrations of 4 μg of inhibitor per ml in combination with standard twofold dilutions of piperacillin.

through production of either the SHV-1 (*K. pneumoniae* GB89) or the SHV-2 (*K. pneumoniae* 192) β-lactamase. Only a single plasmid-mediated β-lactamase was detected in each of the strains. All of the test strains except *E. coli* PABC10 were clinical isolates. Strain *E. coli* PABC10 was a genetic construct in which *E. coli* C600 was transformed with a TEM-10-encoding plasmid. All four isolates were stored at −70°C in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 50% sterile horse serum. Strain purity was confirmed by subculturing freezer stocks onto Trypticase soy agar supplemented with 5% sheep blood (BAP, BBL). Strain *E. coli* PABC10 was also subcultured onto Mueller-Hinton agar (BBL) supplemented with 50 μg of ampicillin per ml for plasmid maintenance.

For in vitro pharmacodynamic studies, logarithmic-phase cultures were prepared by inoculating colonies from an overnight BAP culture into 70 ml of Mueller-Hinton broth (MHB; BBL) to equal an optical density at 540 nm of 0.1. The broth culture was then incubated at 37°C with shaking for 2 h and diluted in sterile MHB to 5×10^5 to 1×10^6 CFU/ml.

Antibiotic preparations. Piperacillin sodium and tazobactam sodium were supplied by Lederle Laboratories, Pearl River, N.Y. Sulbactam sodium was supplied by Roerig-Pfizer, New York, N.Y. Drug powders were reconstituted with phosphate buffer (KH_2PO_4 at 4 g/liter and K_2HPO_4 at 13.6 g/liter) and were sterilized by passage through a 0.20-μm-pore-size Acrodisc syringe filter membrane (Gelman Sciences, Ann Arbor, Mich.).

Antimicrobial susceptibility testing and β-lactamase characterization. Susceptibility tests with piperacillin, tazobactam, sulbactam, piperacillin-tazobactam, and piperacillin-sulbactam were performed by the agar dilution method by the procedure recommended by the National Committee for Clinical Laboratory Standards (13). Susceptibility testing with piperacillin-sulbactam was performed with a fixed 4-μg/ml concentration of sulbactam in combination with standard twofold dilutions of piperacillin. Susceptibility testing with piperacillin-tazobactam was performed with fixed ratios of 8:1 and 4:1 (piperacillin to tazobactam) and with fixed concentrations of 0.5, 1, 2, 4, 8, 16, 32, and 64 μg of tazobactam per ml in combination with standard twofold dilutions of piperacillin. Characterization of β-lactamases included analysis of sonic extracts from cells by isoelectric focusing with known enzyme standards (16) and piperacillin hydrolysis by the microiodometric methodology (14).

IVPM. The IVPM used in these studies was a modification of the original model described by Blaser et al. (2). A hollow-fiber cartridge (Unisyn Fibertech, San Diego, Calif.) was connected by a continuous loop of silicone tubing to a central reservoir. At the start of each experiment, peak antibiotic concentrations in the central reservoir were pumped through the hollow fibers of the cartridge and back into the central reservoir. As drug-containing MHB passed through the hollow fibers, the pores in the fiber walls allowed diffusion of drug from the lumen of the fibers into the peripheral compartment of the cartridge. A comparison of piperacillin and inhibitor levels between the central reservoir and the peripheral compartment every 15 min after dosing demonstrated that equilibrium was established between the compartments at approximately 0.5 h. The exclusion size (molecular weight, 30,000) of the pores in the fiber walls prohibited the bacteria introduced into the peripheral compartment from entering the hollow fibers. Thus, the drug concentration in the peripheral compartment could be altered without disrupting bacterial growth.

The elimination pharmacokinetics of piperacillin, tazobactam, and sulbactam in humans were simulated in the peripheral compartment by a process of dilution and elimination of drug in the central reservoir. The drug concentrations in the central reservoir (and in the peripheral compartment as equilibrium was maintained) were decreased by the addition of drug-free MHB from a dilution reservoir. To maintain a constant volume in the central reservoir, drug-containing MHB was pumped from the central reservoir into an elimination reservoir. The rate at which drug was eliminated from the central reservoir and peripheral compartment by this method was determined by the flow rate of the peristaltic pumps. This rate was calculated from an equation for clearance by monoexponential decline based on the half-life of the piperacillin, tazobactam, or sulbactam in humans and the volume of medium in the central reservoir. An elimination half-life of 1 h for piperacillin, tazobactam, and sulbactam was simulated by this method.

Pharmacokinetics of piperacillin-tazobactam and piperacillin-sulbactam in the IVPM. Peak levels of piperacillin-tazobactam and piperacillin-sulbactam observed in human serum after intravenous administration of 3 g of piperacillin, 0.375 g of tazobactam, and 0.5 g of sulbactam were simulated in the IVPM. The doses of 0.375 g of tazobactam and 0.5 g of sulbactam were chosen since they provide similar pharmacokinetic profiles in humans (9, 15, 18). For simultaneous dosing regimens, both piperacillin and inhibitor were introduced into the central compartment at 0 h. For sequential dosing regimens, tazobactam or sulbactam was administered at 0 h and piperacillin was dosed 0.5 h later, coinciding with the observed peak of inhibitor in the peripheral compartment. To evaluate the pharmacokinetics of piperacillin, tazobactam, and sulbactam in the IVPM, samples were removed from the peripheral compartment at 0, 0.5, 1, 2, 3, 4, and 6 h after dosing into the central compartment, and drug concentrations were measured by bioassay. A penicillinase-negative *Staphylococcus aureus* strain was used for the piperacillin bioassay, and *Acinetobacter calcoaceticus* was used for both tazobactam and sulbactam. Samples assayed for tazobactam and sulbactam were first treated for 20 min with a Bush group 1 cephalosporinase (type III; Sigma Chemical Co., St. Louis, Mo.) to inactivate the piperacillin. Preliminary tests indicated that this treatment did not significantly alter tazobactam or sulbactam concentrations.

Pharmacodynamics of piperacillin-tazobactam and piperacillin-sulbactam in the IVPM. Logarithmic-phase cultures of each test strain were introduced into the peripheral compartment of the IVPM and were exposed to piperacillin-tazobactam or piperacillin-sulbactam as described above. At 0, 0.5, 1, 2, 3, 4, and 6 h, 400 μl of each of the samples taken from the peripheral compartment was treated for 15 min with 100 μl of concentrated penicillinase from culture supernatants of *Bacillus cereus* (BBL) to inactivate the piperacillin. Viable bacterial counts were measured by plating serial 10-fold dilutions of each sample into Mueller-Hinton agar (BBL) and incubating overnight at 37°C. The lowest dilution plated was 0.1 ml of undiluted sample from the peripheral compartment. Since 30 colonies is the lower limit of accurate quantitation by the pour plate methodology, the lowest number of bacteria that could be accurately counted was 300 CFU/ml. The lowest level of detection of viable cells was 10 CFU/ml, although actual counts were inaccurate.

Samples taken at 0.5 h after dosing (observed pharmacokinetic peak) were filter sterilized to remove bacteria, and piperacillin, tazobactam, and sulbactam concentrations were measured by bioassay to ensure that peak levels were within the desired range. Preliminary experiments indicated that the pharmacokinetics of piperacillin, tazobactam, and sulbactam in the peripheral compartment were unaffected by the introduction of the challenge strains into the IVPM except at time points when bacterial counts exceeded 10^7 CFU/ml. Therefore, drug levels were not measured at other time points during the pharmacodynamic experiments.

RESULTS

Characterization of experimental strains. Tables 1 and 2 summarize the β-lactamase characteristics of the test panel, as well as the MICs of piperacillin, tazobactam, sulbactam, piperacillin-tazobactam, and piperacillin-sulbactam for these strains. Piperacillin resistance (>512 μg/ml) was mediated by the production of either the TEM-1 or the TEM-10 β-lactamase in the *E. coli* strains or the SHV-1 or the SHV-2 β-lactamase in *K. pneumoniae* strains (Table 1). Only one type of plasmid-mediated β-lactamase was detected in each strain.

Piperacillin-tazobactam (with a constant 4 μg of tazobactam per ml) and piperacillin-sulbactam (with a constant 4 μg of sulbactam per ml) were inactive at concentrations below 256 μg/ml against the two *K. pneumoniae* isolates (Table 1). For *E. coli* PABC10, the MIC of piperacillin-sulbactam was at least

TABLE 2. Susceptibilities of test strains to piperacillin-tazobactam

Strain	Piperacillin-tazobactam MIC (μg/ml)[a]									
	8:1 ratio	4:1 ratio	0.5 μg/ml	1 μg/ml	2 μg/ml	4 μg/ml	8 μg/ml	16 μg/ml	32 μg/ml	64 μg/ml
E. coli MISC119	16/2	8/2	64/0.5	16/1	2/2	0.25/4	≤0.06/8	≤0.06/16	≤0.06/32	≤0.06/64
E. coli PABC10	4/0.5	2/0.5	2/05	0.5/1	≤0.06/2	≤0.06/4	≤0.06/8	≤0.06/16	≤0.06/32	≤0.06/64
K. pneumoniae GB89	256/32	128/32	>256/0.5	>256/1	>256/2	>256/4	256/8	256/16	256/32	64/64
K. pneumoniae 192	>512/64	256/64	>256/0.5	>256/1	>256/2	>256/4	>256/8	>256/16	>256/32	256/64

[a] Agar dilution susceptibility testing was performed by using the procedures recommended by the National Committee for Clinical Laboratory Standards (13). For piperacillin-tazobactam, tests were performed with fixed ratios of 8:1 and 4:1 (piperacillin to tazobactam), or a fixed concentration of 0.5, 1, 2, 4, 8, 16, 32, or 64 μg of tazobactam per ml was combined with standard twofold dilutions of piperacillin.

fourfold above the MIC of piperacillin-tazobactam, and for *E. coli* MISC119, the MIC of piperacillin-sulbactam was 512-fold higher than the MIC of piperacillin-tazobactam.

When piperacillin-tazobactam MICs obtained with a fixed 8:1 ratio were compared with those obtained with a fixed 4:1 ratio, the tazobactam component at the MIC remained constant by both methods, whereas the piperacillin component consistently differed twofold between the methodologies (Table 2). This suggested that the tazobactam component may be more important than the piperacillin component for determining the MIC at these ratios, and that some minimum critical concentration (MCC) of tazobactam was identified by both tests. In tests with fixed concentrations of tazobactam with standard twofold dilutions of piperacillin (Table 2), the amount of piperacillin required to maintain antibacterial activity over 18 h increased significantly when the concentration of tazobactam was decreased below the MCC identified by the fixed ratio tests. Furthermore, the concentration of piperacillin required to maintain antibacterial activity over 18 h decreased significantly when the concentration of tazobactam was above the MCC identified by the fixed ratio tests.

Pharmacokinetics of piperacillin-tazobactam and piperacillin-sulbactam. The pharmacokinetic profiles of piperacillin-tazobactam and piperacillin-sulbactam dosed simultaneously and sequentially into the IVPM are shown in Fig. 1. Because the pharmacokinetic profiles of 0.375 g of tazobactam and 0.5 g of sulbactam were similar, pharmacokinetic data for these inhibitors were averaged together to provide one curve in each

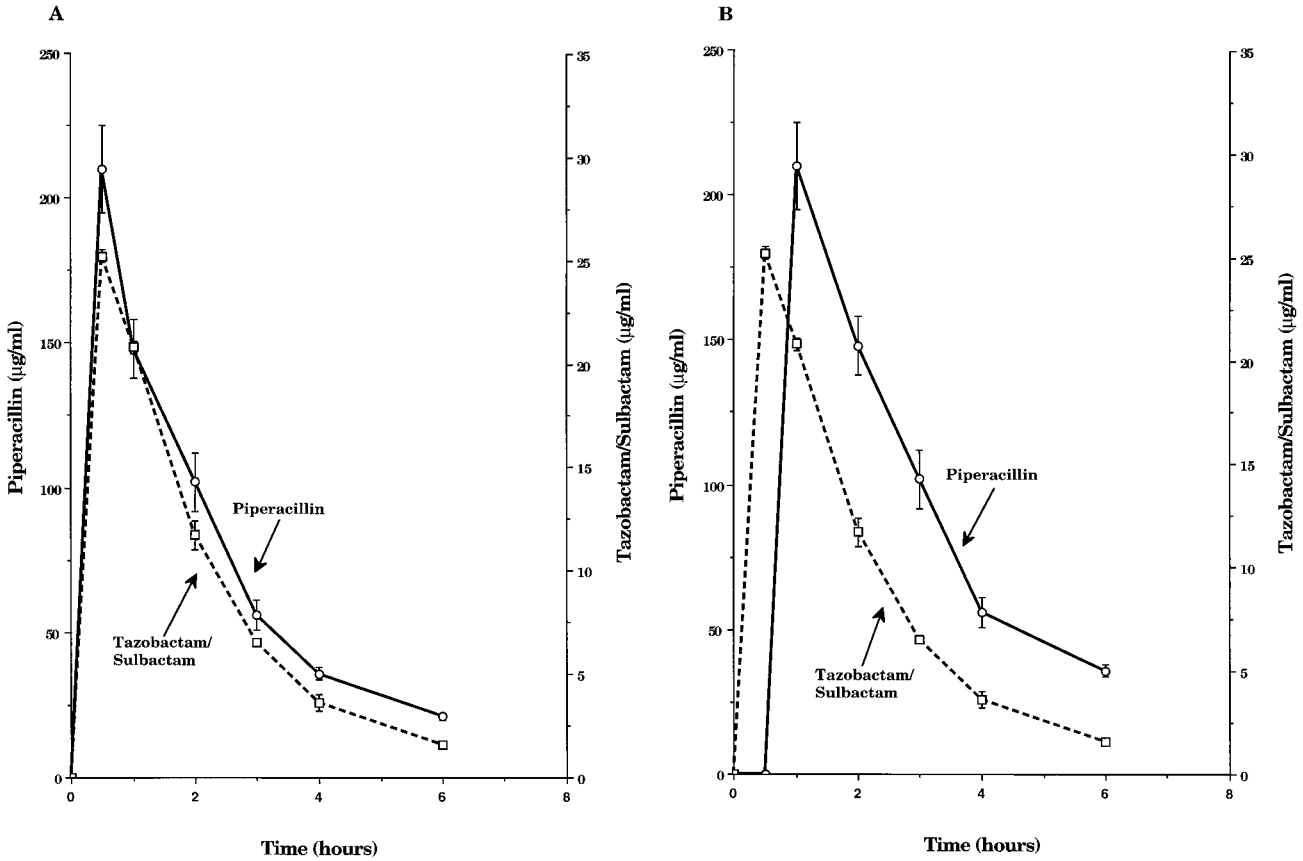

FIG. 1. Pharmacokinetics of piperacillin, tazobactam, and sulbactam in the peripheral compartment of the IVPM after dosing peak concentrations of piperacillin-tazobactam and piperacillin-sulbactam into the central reservoir. Data are for simulated 3.0-g doses of piperacillin administered simultaneously (A) or sequentially (B) with 0.375 g of tazobactam or 0.5 g of sulbactam. Drug levels were measured by bioassay. Each datum point for piperacillin represents the mean drug level in the peripheral compartment (in micrograms per milliliter) for three experimental runs. Each datum point for tazobactam or sulbactam represents the mean concentration in the peripheral compartment (in micrograms per milliliter) for six experimental runs, or three experimental runs for each inhibitor. Error bars indicate standard errors.

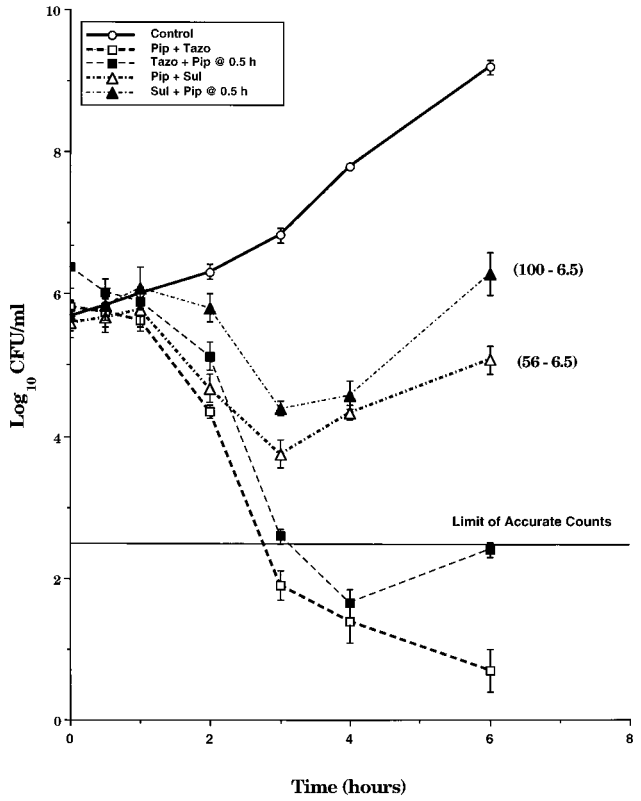

FIG. 2. Time-kill pharmacodynamics of simultaneous versus sequential dosing of piperacillin-tazobactam and piperacillin-sulbactam against *E. coli* MISC119 producing TEM-1. For simultaneous dosing regimens, piperacillin was dosed at 0 h with tazobactam (Pip + Tazo) or sulbactam (Pip + Sul). For the sequential dosing regimens, piperacillin was dosed 0.5 h after dosing with tazobactam (Tazo + Pip @ 0.5 h) or sulbactam (Sul + Pip @ 0.5 h). Each datum point represents the mean CFU per milliliter of MHB from the peripheral compartment for duplicate experiments. Error bars indicate standard errors. Numbers in parentheses represent the mean concentrations of piperacillin-sulbactam (in micrograms per milliliter) at the time point when antibacterial activity ceased.

panel of Fig. 1. The individual peak levels (mean ± standard error of the mean) of piperacillin, tazobactam, and sulbactam achieved were 215 ± 15, 25 ± 1, and 24 ± 2 μg/ml, respectively. In the simultaneous dosing regimen (Fig. 1A), peak concentrations of piperacillin and inhibitor in the peripheral compartment were observed at 0.5 h. In the sequential dosing regimen (Fig. 1B), peak levels of piperacillin were observed at 1 h instead of 0.5 h. The calculated half-lives for piperacillin, tazobactam, and sulbactam in the IVPM were 57 ± 3, 61 ± 4, and 60 ± 2 min, respectively.

Pharmacodynamics against *E. coli* producing TEM β-lactamases. In studies with *E. coli* MISC119 (TEM-1), the pharmacodynamics observed after sequential dosing of piperacillin-tazobactam were similar to those observed after simultaneous administration (Fig. 2). Following a 1-h delay in killing, both regimens rapidly decreased viable bacterial counts 4 logs over the next 3 h and maintained bacterial counts below the limit of accurate counts throughout the remainder of the dosing interval. The pharmacodynamics observed with piperacillin-sulbactam differed markedly from those observed with piperacillin-tazobactam (Fig. 2). Both the simultaneous and sequential piperacillin-sulbactam dosing regimens decreased viable bacterial counts 1 to 1.5 logs over 3 h, followed by 1 to 2 logs of regrowth. The concentration of sulbactam in the peripheral compartment at the time when antibacterial activity ceased was approximately 6.5 μg/ml for both regimens (Fig. 2). Piperacillin concentrations at the same time point were approximately 56 μg/ml for the simultaneous regimen and 100 μg/ml for the sequential regimen.

In studies with *E. coli* PABC10 (TEM-10), both piperacillin combinations decreased viable bacterial counts 3 to 4 logs over the entire 6-h dosing interval (Fig. 3). No differences in pharmacodynamics were observed between the simultaneous and sequential dosing of these combinations.

Pharmacodynamics against *K. pneumoniae* producing SHV β-lactamases. In studies with *K. pneumoniae* GB89 (SHV-1), the pharmacodynamics of piperacillin-tazobactam were similar whether the combination was dosed simultaneously or in sequence (Fig. 4). Following a 1-h delay in killing, both simultaneous and sequential dosing regimens decreased viable bacterial counts 1 log over 3 h. A rapid 2- to 3-log regrowth of the inoculum was then observed over the remainder of the dosing interval. The concentration of tazobactam in the peripheral compartment at the time when antibacterial activity ceased was approximately 6.5 μg/ml for both regimens (Fig. 4). Piperacillin concentrations at the same time point were approximately 56 μg/ml for the simultaneous regimen and 100 μg/ml for the sequential regimen.

The pharmacodynamics of piperacillin-sulbactam differed markedly from those of piperacillin-tazobactam (Fig. 4). Both

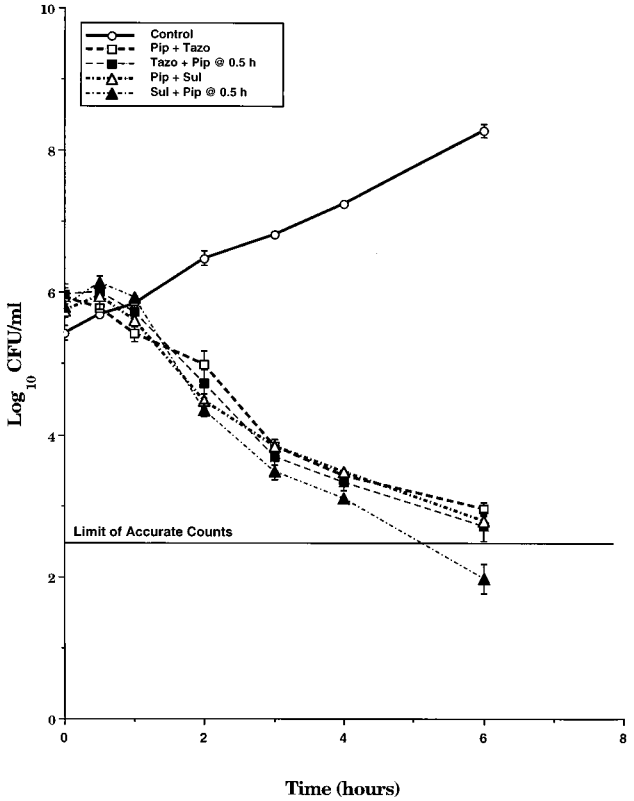

FIG. 3. Time-kill pharmacodynamics of simultaneous versus sequential dosing of piperacillin-tazobactam and piperacillin-sulbactam against *E. coli* PABC10 producing TEM-10. For simultaneous dosing regimens, piperacillin was dosed at 0 h with tazobactam (Pip + Tazo) or sulbactam (Pip + Sul). For sequential dosing regimens, piperacillin was dosed 0.5 h after dosing with tazobactam (Tazo + Pip @ 0.5 h) or sulbactam (Sul + Pip @ 0.5 h). Each datum point represents the mean CFU per milliliter of MHB from the peripheral compartment for duplicate experiments. Error bars indicate standard errors.

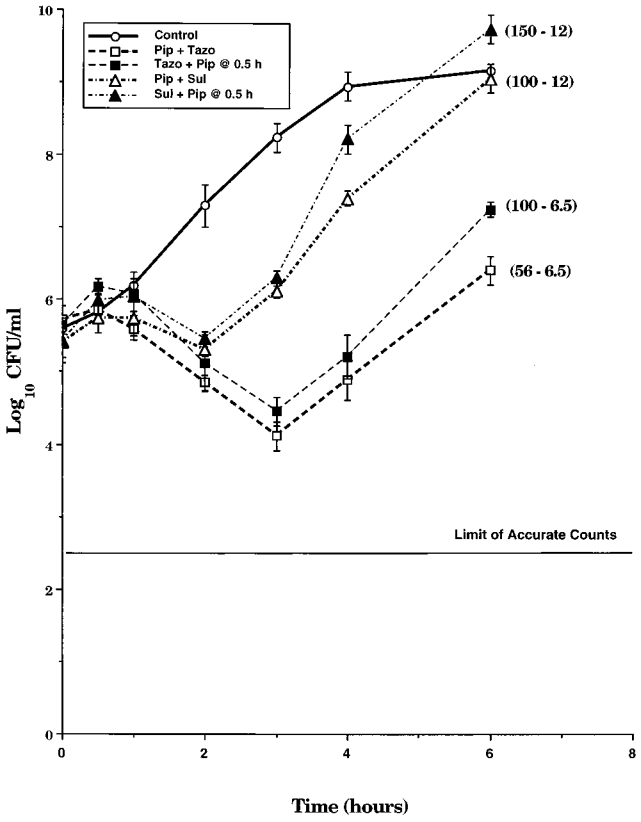

FIG. 4. Time-kill pharmacodynamics of simultaneous versus sequential dosing of piperacillin-tazobactam and piperacillin-sulbactam against *K. pneumoniae* GB89 producing SHV-1. For simultaneous dosing regimens, piperacillin was dosed at 0 h with tazobactam (Pip + Tazo) or sulbactam (Pip + Sul). For sequential dosing regimens, piperacillin was dosed 0.5 h after dosing with tazobactam (Tazo + Pip @ 0.5 h) or sulbactam (Sul + Pip @ 0.5 h). Each datum point represents the mean CFU per milliliter of MHB from the peripheral compartment for duplicate experiments. Error bars indicate standard errors. Numbers in parentheses represent the mean concentrations of piperacillin-tazobactam or piperacillin-sulbactam (in micrograms per milliliter) at the time point when antibacterial activity ceased.

the simultaneous and sequential dosing regimens showed little killing during the first 2 h of the dose interval, which was followed by a rapid 4-log increase in bacterial counts. The concentration of sulbactam at the time when antibacterial activity ceased was approximately 12 μg/ml for both regimens (Fig. 4). Piperacillin concentrations at this time point were approximately 100 μg/ml for the simultaneous regimen and 150 μg/ml for the sequential regimen.

In studies with *K. pneumoniae* 192 (SHV-2), the pharmacodynamics of piperacillin-tazobactam and piperacillin-sulbactam were similar. Both combinations were bacteriostatic for 2 h, followed by growth of the inoculum over the remainder of the dose interval. The concentrations of sulbactam and tazobactam at the time when antibacterial activity ceased were approximately 12 μg/ml for all regimens (Fig. 5). The coinciding concentrations of piperacillin were 100 μg/ml for the simultaneous regimens and 150 μg/ml for the sequential regimens.

DISCUSSION

By using an IVPM, certain pharmacodynamic principles governing the antibacterial activity of piperacillin-tazobactam and piperacillin-sulbactam against β-lactamase-producing *E. coli* and *K. pneumoniae* were elucidated. In experiments with all four challenge strains, the pharmacodynamics observed when piperacillin was dosed 0.5 h after dosing with tazobactam or sulbactam were similar to those observed when the combinations were dosed simultaneously. Sequential dosing, therefore, neither enhanced nor diminished the bactericidal activities of the combinations. Since bacteria in the sequential dosing studies were exposed to a constant fluctuation in the ratio of piperacillin to β-lactamase inhibitor, in contrast to the constant 8:1 ratio in the simultaneous regimen studies, these data suggest that the bactericidal activities of piperacillin-tazobactam and piperacillin-sulbactam were not dependent upon the maintenance of a constant 8:1 ratio between the components. This conclusion is supported by data from similar studies with ampicillin-sulbactam against TEM-1-producing *E. coli* (1) and by in vitro studies with a combination of ceftriaxone and sulbactam against an *E. coli* strain producing an SHV-2 enzyme (8). Using static time-kill methodologies, Fantin et al. (8) demonstrated that the pharmacodynamics of ceftriaxone-sulbactam combinations were similar whether 4 or 8 μg of ceftriaxone per ml was combined with the same concentration of sulbactam. Interestingly, when these studies were extended to an animal model of infection, the level of killing observed with a dose of ceftriaxone of 30 mg/kg of body weight combined with a 100-mg/kg dose of sulbactam was significantly increased compared

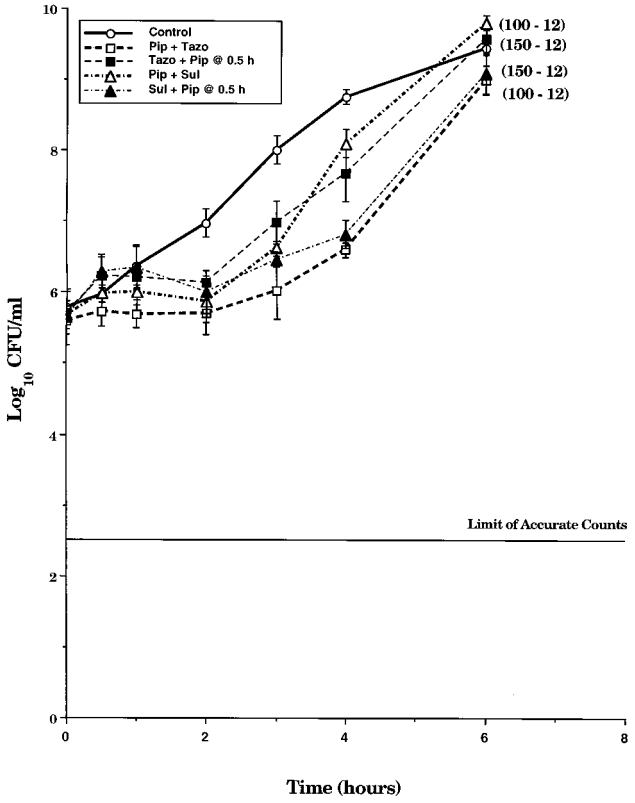

FIG. 5. Time-kill pharmacodynamics of simultaneous versus sequential dosing of piperacillin-tazobactam and piperacillin-sulbactam against *K. pneumoniae* 192 producing SHV-2. For simultaneous dosing regimens, piperacillin was dosed at 0 h with tazobactam (Pip + Tazo) or sulbactam (Pip + Sul). For sequential dosing regimens, piperacillin was dosed 0.5 h after dosing with tazobactam (Tazo + Pip @ 0.5 h) or sulbactam (Sul + Pip @ 0.5 h). Each datum point represents the mean CFU per milliliter of MHB from the peripheral compartment for duplicate experiments. Error bars indicate standard errors. Numbers in parentheses represent the mean concentrations of piperacillin-tazobactam or piperacillin-sulbactam (in micrograms per milliliter) at the time point when antibacterial activity ceased.

to the level of killing observed with a 15-mg/kg dose of ceftriaxone and the same 100-mg/kg dose of sulbactam (8). Thus, the in vivo data of this study contradict the in vitro kinetic data. Although the in vivo data showed a difference between the two doses, it is difficult to determine whether the increased killing observed with 30 mg of ceftriaxone per kg was the result of a more optimal ratio between ceftriaxone and sulbactam or was the result of higher levels of ceftriaxone in the animals compared to the levels in those dosed with 15 mg/kg. In the current study, the amounts and concentrations of piperacillin and inhibitor delivered over the dose interval were essentially the same for both regimens, such that the most important variable between simultaneous and sequential dosing regimens was the constant fluctuations in the ratio between components after sequential dosing. Therefore, the model used in this and the previous study with ampicillin-sulbactam (1) appears to more accurately address the role of maintaining a critical ratio between components on the pharmacodynamics of inhibitor-drug combinations.

In experiments when regrowth of the inoculum was observed, increases in bacterial counts initiated at the same time point whether the piperacillin-inhibitor combinations were dosed simultaneously or sequentially. Since the concentration of piperacillin present in the sequential regimen was essentially twice that present in the simultaneous regimen at the start of regrowth, while the concentrations of inhibitors were the same, antibacterial activity appeared to be lost when inhibitor levels fell below some critical concentration. These data suggest that when the piperacillin-inhibitor combinations were dosed at a ratio of 8:1, the pharmacokinetics of the inhibitors were more critical than those of piperacillin in dictating the length of time that the antibacterial activity was maintained after dosing. This conclusion is supported by data from similar studies with ampicillin-sulbactam against *E. coli* strains producing TEM-1 (1) and by data from other investigators (6, 11). Using a similar IVPM, Dudley et al. (6) demonstrated that lower doses of piperacillin combined with usual doses of tazobactam provided bactericidal activity against piperacillin-resistant *E. coli* comparable to the bactericidal activity that was observed against an isogenic piperacillin-susceptible strain. However, when the pharmacokinetics of tazobactam were altered by dosing at 12-h rather than 8-h intervals, activity was diminished, even if the dose of piperacillin was doubled. In an in vivo study of different piperacillin-tazobactam regimens in the treatment of experimental meningitis with a TEM-3-producing strain of *K. pneumoniae*, Leleu et al. (11) concluded that the concentration of tazobactam in cerebrospinal fluid, but not that of piperacillin, was the important limiting factor determining the activity of the combination (11). Taken together, these studies highlight the importance of tazobactam and sulbactam pharmacokinetics over those of piperacillin and ampicillin on the bactericidal activity and pharmacodynamics of piperacillin-tazobactam and ampicillin-sulbactam.

The relative importance of β-lactamase inhibitor kinetics over those of the β-lactams in inhibitor-drug combinations is further supported by the MIC data obtained in this study with piperacillin-tazobactam and in a previous study with ampicillin-sulbactam (1). These studies have shown that in tests performed with fixed ratios of inhibitor-drug combinations, the MICs obtained were more dependent on the amount of inhibitor present than the amount of β-lactam present. Furthermore, evaluation of the MICs obtained with a range of fixed inhibitor concentrations demonstrated that the amount of β-lactam required at the MIC was significantly dependent on the amount of inhibitor used, whereas the amount of inhibitor at the MIC was relatively independent of the amount of β-lactam present.

It is evident from these studies that with the current dosing of piperacillin-tazobactam at an 8:1 ratio and ampicillin-sulbactam at a 2:1 ratio, antibacterial activity will be lost at a time point when inhibitor concentrations fall below some critical concentration. This critical concentration is most likely the amount of inhibitor required to maintain continued suppression of β-lactamase in the presence of continuous enzyme production and inhibitor turnover and appears to be dependent on the amount and type of enzyme produced by the target bacterium, as well as the potency of the inhibitor in the combination. With increasing levels of enzyme production, the minimum amount of inhibitor required for suppression would be expected to increase and antibacterial activity would be expected to cease sooner. Although only four strains were evaluated in this study, a comparison of β-lactamase levels from Table 1 with the length of time that antibacterial activity was maintained does suggest an inverse relationship between these two parameters. The level of enzyme production, however, was not the only parameter influencing the pharmacodynamics of the piperacillin-inhibitor combinations in this study. A second important factor was the type of enzyme produced by the target bacterium and its susceptibility to inhibition by the β-lactamase inhibitors. In this study, the level of SHV-2 production in *K. pneumoniae* 192 was essentially twice that of SHV-1 produced by *K. pneumoniae* GB89. If the level of enzyme produced was the only important variable influencing the MCC, then initiation of *K. pneumoniae* 192 regrowth with piperacillin-sulbactam would occur before the initiation of *K. pneumoniae* GB89 regrowth. However, the two strains initiated regrowth at the same time point. Analysis of the 50% inhibitory concentrations of sulbactam against SHV-1 and SHV-2 enzymes demonstrates that five- to sixfold less sulbactam is required for 50% reduction of SHV-2 activity compared to the amount required for 50% reduction of SHV-1 activity (3). Therefore, SHV-2 is much more susceptible to the inhibitory activities of sulbactam than SHV-1, counterbalancing the higher levels of enzyme produced by *K. pneumoniae* 192 to the point that the length of time that piperacillin-sulbactam maintained antibacterial activity against the two *K. pneumoniae* strains was similar. In contrast to sulbactam, the activity of tazobactam against SHV-2 and SHV-1 is much more comparable, with 50% inhibitory concentrations differing by only twofold (3). With the two enzymes being more comparable in their susceptibilities to tazobactam inhibition, differences between the two *K. pneumoniae* strains with respect to their levels of enzyme production became a more dominant influence on the pharmacodynamics, and regrowth of *K. pneumoniae* 192 with piperacillin-tazobactam initiated 1 h before regrowth of *K. pneumoniae* GB89. The third important factor influencing the MCC in these pharmacodynamic interactions was the differences in potency between sulbactam and tazobactam. A comparison of piperacillin-tazobactam pharmacodynamics to those of piperacillin-sulbactam against *K. pneumoniae* GB89 and *E. coli* MISC119 showed that regrowth generally initiated sooner in cultures treated with piperacillin-sulbactam than in cultures treated with piperacillin-tazobactam. These data suggest that the amount of sulbactam required to maintain antibacterial activity was higher than that of tazobactam against these strains. This conclusion is supported by in vitro data demonstrating that tazobactam is a more potent inhibitor of TEM-1 and SHV-1 β-lactamases than sulbactam (4); thus, the amount of sulbactam required to maintain antibacterial activity would be expected to be higher and regrowth would be expected to initiate sooner.

Although data from this and the previous study (1) demonstrate the importance of β-lactamase inhibitor pharmacokinetics in dictating the pharmacodynamics of inhibitor-drug combinations over the dosing interval, an important task which remains is determination of which specific pharmacokinetic parameters influence efficacy in the treatment of infections. In a study with piperacillin-tazobactam, Strayer et al. (19) concluded that the area under the concentration curve for β-lactamase inhibitors was the important pharmacokinetic parameter determining the efficacy of inhibitor-drug combinations against β-lactamase-producing bacteria. The relative importance of the area under the concentration curve for tazobactam and sulbactam in this study could not be assessed since the pharmacokinetics of the inhibitors were similar for all dosing regimens. Furthermore, the single-dose design of this study and our previous study with ampicillin-sulbactam (1) did not allow for evaluation of the impact of time above the MCC for the β-lactamase inhibitors on the efficacy of the combination in the treatment of infections. One would expect, however, that time above the MCC and area under the concentration curve for the inhibitors would be directly related. As stated before, increasing levels of enzyme production or the presence of enzymes with decreased susceptibility to inhibition can individually or together increase the amount of β-lactamase inhibitor required to sufficiently suppress enzyme activity. If the total amount of β-lactamase inhibitor delivered over the dose interval is increased as compensation, then one would expect that the time that the inhibitor levels remain above the MCC required for maintenance of antibacterial activity would also increase. Therefore, it is possible that both of these pharmacokinetic parameters are important determinants of the efficacies of inhibitor-drug combinations. However, since the MCC can vary substantially from strain to strain, the area under the concentration curve correlations may be a more accessible parameter to be evaluated, and further investigations into the importance of the area under the concentration curve and other β-lactamase inhibitor kinetics are essential. Regardless of what pharmacokinetic parameter is found to be the most critical for the efficacies of inhibitor-drug combinations, the importance of β-lactamase inhibitor pharmacokinetics in the pharmacodynamic interactions of inhibitor-drug combinations must play a more central role in the design of both susceptibility tests and dosing strategies for these compounds.

In summary, the results of this investigation have demonstrated that one of the most important factors affecting the pharmacodynamics of β-lactamase inhibitor–β-lactam combinations is the maintenance of a critical concentration of inhibitor necessary to sufficiently suppress β-lactamase activity. This critical concentration will vary depending upon the host organism, the amount and type of β-lactamase produced, the specific inhibitor used in the combination, and the pharmacokinetics of the inhibitor over the dose interval. Clearly, additional studies are required to more completely delineate the principles governing the pharmacodynamics of β-lactamase inhibitor–β-lactam drug combinations, especially the influence of different pharmacokinetic parameters of β-lactamase inhibitors on the efficacy of inhibitor-drug combinations. Until the importance of β-lactamase inhibitor pharmacokinetics on the antibacterial activities and efficacies of inhibitor-drug combinations is fully elucidated, questions concerning in vitro susceptibility testing of inhibitor-drug combinations and optimum dosing strategies for these combinations will remain unanswered.

ACKNOWLEDGMENTS

This work was supported by a grant from Lederle Laboratories.

We thank Stacey Edward for excellent technical assistance. We also thank Patricia A. Bradford for the genetic construction of *E. coli* PABC10.

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