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Pharmacodynamics of Piperacillin Alone and in Combination with Tazobactam against Piperacillin-Resistant and -Susceptible Organisms in an In Vitro Model of Infection

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The pharmacodynamics of dosage regimens of piperacillin alone or in combination with tazobactam against piperacillin-resistant or -susceptible bacteria were studied in an in vitro model of infection. Experiments were conducted by using a fixed daily exposure of 12 g of piperacillin, given as 3 g alone or in combination with tazobactam at 0.375 g every 6 h, or the same total dose of the combination given as 4 g of piperacillin plus 0.5 g of tazobactam every 8 h. The addition of tazobactam to piperacillin, irrespective of the dosing interval, did not alter the killing of piperacillin-susceptible organisms (*Escherichia coli* J53 and *Pseudomonas aeruginosa* ATCC 27853). In contrast, experiments with an isogenic TEM-3-containing transconjugant of *E. coli* J53 (*E. coli* J53.2-TEM-3) that was resistant to piperacillin (MIC, 128 μg/ml) showed that the addition of tazobactam resulted in bacterial killing similar to that observed with the wild-type strain. Although tazobactam concentrations fell to less than 4 mg/liter (the concentration associated with a reduction in the piperacillin MIC from 128 to 2 mg/liter) 2 to 3 h after a dose, a similar degree of bacterial killing was observed when the same total 24-h dose of piperacillin-tazobactam was fractionated into dosing intervals of every 6 or 8 h. Investigations with *Staphylococcus aureus* 7176 (piperacillin MIC, 128 μg/ml) showed that the addition of tazobactam, again irrespective of dosing interval, also resulted in net bacterial killing which was not seen with piperacillin alone. These data support the use of extended dosing intervals (every 8 h) of piperacillin-tazobactam in the treatment of infections caused by piperacillin-resistant bacteria.

Piperacillin, an extended-spectrum penicillin, has been used for more than a decade in the treatment of mixed aerobic-anaerobic bacterial infections. The dissemination of β-lactamase-producing bacteria that inactivate piperacillin has decreased the clinical utility of this drug. One strategy used to counteract the problems associated with β-lactamase-producing bacteria has been the development of β-lactamase inhibitors such as clavulanic acid, sulbactam, and most recently, tazobactam (19).

Tazobactam is a triazolymethyl penicillanic acid sulfone which possesses very little intrinsic antibacterial activity (14). When tazobactam is combined with piperacillin, it significantly enhances the in vitro susceptibility of piperacillin against β-lactamase-producing isolates of members of the family *Enterobacteriaceae*, *Staphylococcus* spp., and *Bacteroides* spp. (1, 7, 8, 10, 11, 13, 14, 16, 18, 25).

Although the pharmacokinetic and pharmacodynamic parameters associated with optimal killing of gram-negative aerobic bacteria have been well characterized for β-lactam antibiotics (6), strategies for the design of dosage regimens of β-lactam–β-lactamase inhibitor combinations are not established. The question is relevant since dosage regimens of β-lactamase inhibitor combinations provide concentrations of inhibitor in vivo that exceed the breakpoint concentrations used in in vitro susceptibility testing of combinations for only 2 to 3 h.

The purpose of the study described here was to compare the pharmacodynamics of various dosage regimens of a fixed daily exposure of piperacillin alone and in combination with tazobactam against piperacillin-resistant or -susceptible bacteria in an in vitro model of infection.

MATERIALS AND METHODS

Bacteria. The following strains were studied: *Pseudomonas aeruginosa* ATCC 27853, a stable clinical isolate of methicillin-susceptible *Staphylococcus aureus* 7176 obtained from a patient with bacteremia, and an isogenic pair of *Escherichia coli* strains, the wild type (J53) and the transconjugant possessing the plasmid (J53.2-TEM-3) coding for the TEM-3 extended-spectrum β-lactamase (24).

Susceptibility testing. Table 1 shows the MICs of piperacillin and piperacillin-tazobactam for the test strains. The strains were selected on the basis of their susceptibilities or resistance to piperacillin. *E. coli* J53.2-TEM-3 and *S. aureus* 7176 were piperacillin resistant, but all strains were susceptible to piperacillin-tazobactam (Table 1). MICs were determined by the tube broth macrodilution method with an inoculum of ~5×10^5 CFU/ml by standardized methods (21). The approved susceptibility breakpoints of the National Committee for Clinical Laboratory Standards (22) were used when such criteria existed.

Antibiotics. Piperacillin powder for injection (lot 317-704) and tazobactam analytical powder (lot 6818B46) were supplied by Lederle Laboratories, Pearl River, N.Y.

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TABLE 1. MICs and MBCs for bacteria tested in the model

Strain	Piperacillin MIC/MBC (mg/liter)	
	Piperacillin alone	With tazobactam (4 mg/liter)
E. coli J53	4/16	2/4
E. coli J53.2-TEM-3	128/128	2/2
S. aureus 7176	128/256	2/8
P. aeruginosa ATCC 27853	8/256	8/256

Media. Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.) supplemented with calcium (50 μg/ml) and magnesium (25 μg/ml) (MHB-S) was used for all susceptibility and model experiments.

In vitro model. Hollow-fiber bioreactor chambers (Cell Pharm Mini-Bioreactor System; Unisyn Fibertec Corporation, San Diego, Calif.) were connected in series to a central compartment in an incubator as described previously (17). The volume of each peripheral chamber was 15 ml of 10% heat inactivated human serum–90% MHB-S. The central compartment diluent was MHB-S. Following administration of a dose of piperacillin or piperacillin-tazobactam, drug was eliminated by pumping drug-free MHB-S into the central compartment at a rate adjusted for human pharmacokinetic properties. The elimination half-lives of piperacillin and tazobactam in the in vitro model were adjusted to ~1 h (12).

The bacteria used for all experiments were prepared from inocula grown previously, divided into aliquots, and frozen in a mixture of brain heart infusion broth-fetal calf serum (60:40). On the day of an experiment, the frozen inoculum was thawed, diluted with fresh MHB-S, and incubated for 1.5 h to bring the bacteria into the growth phase. The bacteria were then injected into the peripheral compartments of the model and were incubated for 1 to 1.5 h to reach a target inoculum of $\sim 2 \times 10^6$ CFU/ml.

Dosage regimens. Experiments with piperacillin monotherapy were conducted by using a fixed 24-h exposure of 12 g, with doses given as 3 g every 6 h. Piperacillin-plus-tazobactam experiments used a fixed 24-h exposure of 12 g of piperacillin with 1.5 g of tazobactam. This amount was fractionated into 2 dosage regimens of 3 g of piperacillin plus 0.375 g of tazobactam every 6 h or 4 g of piperacillin plus 0.5 g of tazobactam every 8 h. Doses were given as a simulated intravenous bolus in all regimens.

Pharmacodynamic and pharmacokinetic measurements. Samples (1 to 1.5 ml) were collected from the central and peripheral compartments of the model every 2 to 4 h over a 24-h period. All samples from the peripheral chambers were quantified for the number of bacterial CFU per milliliter. Samples from the central and peripheral compartments of the model were assayed for tazobactam and/or piperacillin content by high-pressure liquid chromatography (23). The lower limits of quantitation for piperacillin and tazobactam were 1 and 10 μg/ml, respectively. The ranges of five standards for each drug were 1 to 50 μg/ml for piperacillin and 10 to 50 μg/ml for tazobactam. The within- and between-day coefficients of variation for three seeded controls of piperacillin (15 to 45 mg/liter) ranged between 7.2 and 10% (20 to 26 determinations of each control). For tazobactam, controls of between 15 and 45 mg/liter had within- and between-day coefficients of variation of between 15 and 22% (7 to 15 determinations for each control). Experimental samples with drug concentrations greater than the ranges of the standards were diluted into the range of the curve. A one-compartment pharmacokinetic model was fit to drug concentrations in the central compartment measured following the first and last doses of both drugs for all regimens by using extended least-squares regression (MKMODEL, version 4; Biosoft, Milltown, N.J.).

Bacterial counts (CFU per milliliter) were determined by serially diluting the sample 10-fold in cold saline and inoculating it (in triplicate) onto drug-free Mueller-Hinton agar (MHA) with subsequent incubation at 37°C for 18 to 24 h. The pharmacokinetics of the drugs in the model and the sampling schedule resulted in little or no carryover of drug. The most drug that was carried over during the processing for CFU per milliliter counts was 7.5 mg of piperacillin per liter in experiments with piperacillin alone against piperacillin-resistant bacteria; in view of the high MICs for these strains (128 mg/liter), this amount would have a negligible effect on the recovery of bacteria. Small numbers of bacteria (i.e., ≤1,000 CFU/ml) were counted by placing 100 μl of sample into ~10 ml of cold saline and filtering this mixture through a 0.45-μm-pore-size filter (Millipore Corporation, Bedford, Mass.). The filter was washed several times with sterile saline and was then placed directly onto MHA and incubated, and the colonies were counted; thus, the theoretical limit of detection for this method is 10 CFU/ml. The dilution of the sample into saline and washing with an additional 5 to 10 ml makes it unlikely that significant amounts of drug are transferred with bacteria on the filter to MHA. In all experiments, samples collected at 0 and 24 h were also processed on MHA containing 128 μg of piperacillin per ml and 128 and 4 μg of piperacillin-tazobactam per ml, respectively, to quantify drug-resistant bacterial subpopulations. The MICs for all strains were determined before the model experiments and for posttreatment isolates (24 h) recovered on drug-free MHA plates.

Post-β-lactamase inhibition effect. In separate experiments with static drug concentrations, a ca. 10^6 inoculum of growing *E. coli* J53.2-TEM-3 was exposed for 2 h to the integral mean (area under the concentration-time curve from 0 to 2 h [AUC_{0-2}]/2) concentrations of drug obtained in the model of tazobactam alone or tazobactam combined with piperacillin. Following a 2-h exposure, the bacteria were washed three times and were resuspended in prewarmed medium containing either piperacillin alone or piperacillin combined with tazobactam at mean integral concentrations of drug simulated in the model 2 to 8 h after a dose (i.e., AUC_{2-8}/6). Samples were taken hourly and were processed for bacterial counts as described above.

RESULTS

Pharmacokinetics. Figure 1 depicts the simulated and measured piperacillin and tazobactam concentrations in the central compartment of the model. The mean piperacillin concentrations of samples collected simultaneously from the central and peripheral compartments of the model were within 5 to 20% of each other, indicating rapid equilibration between the central and peripheral compartments. Mean piperacillin pharmacokinetic parameters are displayed in Table 2. In experiments that assessed the pharmacodynamics of piperacillin given as 3 g every 6 h against piperacillin-resistant bacteria, the piperacillin concentrations measured in the central and peripheral compartments were lower than targeted concentrations but still within acceptable ranges (data not shown). This was expected, however, because of bacterial production of β-lactamase and the inactivation of piperacillin, which led to "sink" conditions in the model.

For the test strains susceptible to piperacillin alone or piperacillin combined with tazobactam, piperacillin concentra-

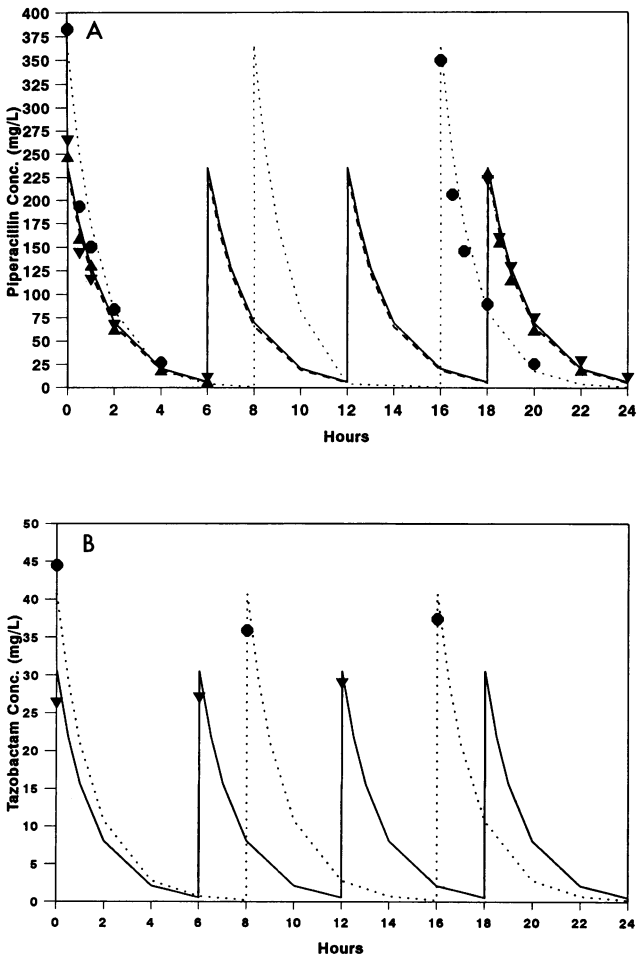

FIG. 1. Simulated (lines) and measured (points) concentrations of piperacillin (A) and tazobactam (B) in the central compartment of the in vitro model. Simulated concentrations for piperacillin were derived by using pharmacokinetic parameters generated from fits of individual experiments for which the data are given in Table 2, whereas tazobactam concentrations were generated from values reported in the literature. The measured drug concentrations are means for two to four samples. The regimens were piperacillin given as 3 g every 6 h (▲), piperacillin and tazobactam given as 3 and 0.375 g, respectively, every 6 h (▼), and piperacillin and tazobactam given as 4 and 0.5 g, respectively, every 8 h (●).

tions exceeded the MIC for 63 to 100% of a 24-h dosing cycle with all regimens (Table 2). In contrast, experiments with piperacillin alone against piperacillin-resistant bacteria (i.e., *E. coli* J-53-TEM-3 and *S. aureus* 7176) resulted in concentrations of piperacillin greater than the starting MICs (128 mg/liter) for only 17% of a 24-h period.

Mean peak tazobactam concentrations in the central compartment for 3 g of piperacillin plus 0.375 g of tazobactam every 6 h and 4 g of piperacillin plus 0.5 g of tazobactam were 27.4 and 39.0 μg/ml, respectively. The mean elimination half-lives for 3 g of piperacillin plus 0.375 g of tazobactam every 6 h and 4 g of piperacillin plus 0.5 g of tazobactam were 1.3 and 1.2 h, respectively. The concentrations of tazobactam exceeded 4 mg/liter for ≤50% of a 24-h dosing cycle with either regimen.

Pharmacodynamics. All bacterial strains tested in the model grew well during control experiments (no drug exposure).

(i) *E. coli* J53. The first dose of all regimens resulted in a marked bactericidal effect; on average, a 4-log-unit reduction in the number of CFU per milliliter occurred within the first dosing interval (Fig. 2A). Mean bacterial counts of ≤10 CFU/ml were achieved with all regimens by the end of the second dosing interval. There was no difference in bacterial killing with either regimen of piperacillin-tazobactam from that observed with piperacillin monotherapy.

Experiments with piperacillin alone against *E. coli* J53-TEM-3 showed the effect of the inactivating enzyme on pharmacodynamic properties (Fig. 2B). Although *E. coli* J53-TEM-3 was resistant, the piperacillin MIC for the strain was exceeded in the model for ~2 h, thus resulting in only transient bacterial killing and then the regrowth of bacteria fully resistant to the piperacillin concentrations obtained in the model. The numbers of bacteria recovered on drug-free MHA and MHA with 128 mg of piperacillin per liter were identical.

The addition of tazobactam to piperacillin restored the bacterial killing of the β-lactamase-producing isogenic strain of *E. coli* (J53.2-TEM-3) by piperacillin to that observed with the parent strain (Fig. 2B). There was no difference in bacterial killing when the fixed daily exposure of piperacillin-tazobactam was fractionated into dosing intervals of every 6 or 8 h. When samples collected at 24 h from the piperacillin monotherapy experiment were grown on drug-containing MHA to quantify drug-resistant subpopulations, the entire inoculum was resistant to 128 μg of piperacillin per ml; however, no growth was seen on plates containing 128 and 4 μg of piperacillin and tazobactam per ml, respectively. The MICs for posttreatment bacteria (24 h) recovered on drug-free MHA were unchanged.

Experiments with static concentrations of drugs designed to mimic the same time-averaged exposure did not demonstrate a post-β-lactamase inhibition effect; bacterial killing occurred only when tazobactam was present in the culture at the same time as piperacillin.

(ii) *S. aureus* 7176. Piperacillin monotherapy resulted in approximately a 1.5-log-unit reduction in the number of CFU

TABLE 2. Piperacillin and tazobactam pharmacokinetic parameters in central compartment of in vitro model according to regimen

Drug and parameter[a]	Piperacillin (3 g) every 6 h	Piperacillin (3 g) + tazobactam (0.375 g) every 6 h	Piperacillin (4 g) + tazobactam (0.5 g) every 8 h
Piperacillin			
AUC (mg · h/liter)[b]	366.7	388.2	488.9
C_{max} (mg/liter)	227.6	235.7	366.7
$t_{1/2}$ (h)	1.2	1.3	1.3
% Time > MIC[c]			
E. coli J-53	100	100	83
E. coli J-53-TEM-3	17	100	83
P. aeruginosa 27853	83	83	63
S. aureus 7176	17	100	83
Tazobactam			
AUC (mg · h/liter)[b]	NA[d]	42.5	64.7
C_{max} (mg/L)	NA	27.6	36.5
$t_{1/2}$ (h)	NA	1.3	1.3
% Time > 4 mg/liter	NA	50	38

[a] All parameters for piperacillin and the maximum concentration of tazobactam in serum are based on measured concentrations; the other tazobactam parameters are based on target simulations. AUC, area under the concentration-time curve; C_{max}, maximum concentration of drug in serum; $t_{1/2}$, half-life.
[b] Per dose.
[c] Percentage of 24-h period that drug concentrations exceeded the value shown for each regimen.
[d] NA, not applicable.

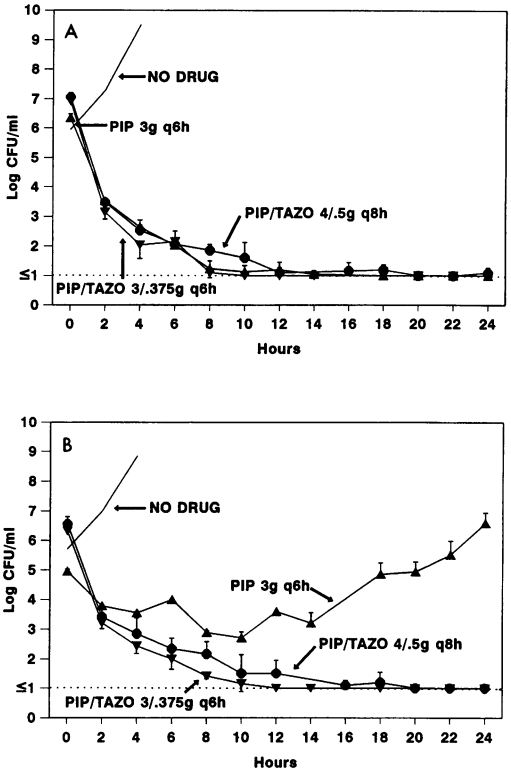

FIG. 2. Pharmacodynamics of piperacillin (PiP) alone or in combination with tazobactam (TAZO) against *E. coli* J53 (A) and *E. coli* J53.2-TEM-3 (B) in an in vitro model of infection. Datum points are geometric means ± standard deviations for three to six within-day replications. The regimens were piperacillin given as 3 g every 6 h (q6h) (▲), piperacillin and tazobactam given as 3 and 0.375 g, respectively, every 6 h (q6h) (▼), and piperacillin and tazobactam given as 4 and 0.5 g, respectively, every 8 h (q8h) (●).

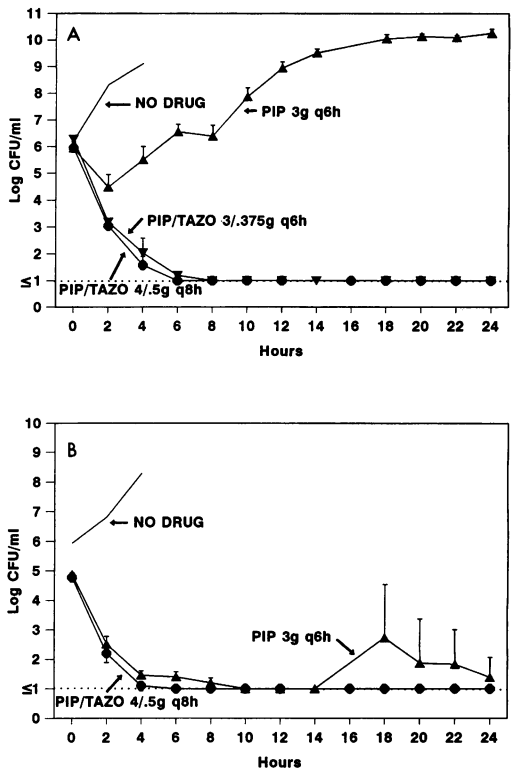

FIG. 3. Pharmacodynamics of piperacillin (PiP) alone or in combination with tazobactam (TAZO) against *S. aureus* 7176 (A) and *P. aeruginosa* ATCC 27853 (B). The regimens were piperacillin given as 3 g every 6 h (q6h) (▲), piperacillin and tazobactam given as 3 and 0.375 g, respectively, every 6 h (q6h) (▼), and piperacillin and tazobactam given as 4 and 0.5 g, respectively, every 8 h (q8h) (●).

per milliliter over 2 h; this was followed by bacterial regrowth (Fig. 3A). In contrast, both piperacillin-tazobactam regimens were highly active against this strain. There was no difference in bacterial killing when the fixed daily exposure of piperacillin-tazobactam was fractionated into dosing intervals of every 6 and 8 h. Subpopulation analysis and the posttreatment MICs obtained from the piperacillin monotherapy experiment were comparable to the results obtained for the other β-lactamase-producing strain.

(iii) *P. aeruginosa* ATCC 27853. All regimens of piperacillin alone or combined with tazobactam resulted in a 4-log-unit reduction in the number of CFU per milliliter during the first dosing interval (Fig. 3B). As was seen with *E. coli* J53, the addition of tazobactam had no effect on piperacillin pharmacodynamics. Killing was fairly consistent between regimens until the 14-h time point, when 2 log units of bacterial regrowth occurred with the piperacillin monotherapy regimen. When samples collected at 24 h from the piperacillin monotherapy experiment were grown on MHA containing 128 μg of piperacillin per ml no growth occurred. The MICs for posttreatment bacteria recovered on drug-free MHA were unchanged.

DISCUSSION

When using β-lactamase inhibitor combinations, it is important that an adequate amount of inhibitor be provided in vivo to inactivate the β-lactamases produced by actively growing bacteria at the site of infection. Livermore (15) has proposed that the activities of various β-lactam–β-lactamase inhibitor combinations be determined by assessment of the concentration of inhibitor required to bring the susceptibilities of individual organisms to below accepted susceptibility breakpoints for the β-lactam alone or even to the susceptibilities for β-lactamase-negative, wild-type strains. Since inactivation of β-lactamases follows stoichiometry on the basis of turnover of the enzyme, the amount of β-lactamase inhibitor necessary to augment the activity of a β-lactam in vivo would be based on the provision of an adequate amount of inhibitor provided over time, or the AUC. Failure to deliver adequate amounts of inhibitor would be expected to result in resistance to the drug combination, including bacterial regrowth at the end of a dosage interval. Resistance to β-lactam–β-lactamase inhibitor combinations has been described in certain strains of *E. coli* that hyperproduce the TEM-1 β-lactamase, and hence have a relative insufficiency of inhibitor (15, 16).

The results of the present study indicate that a simulated fixed daily exposure of piperacillin-tazobactam given as divided doses every 6 or 8 h results in similar pharmacodynamic effects against a piperacillin-resistant *E. coli* strain and *S. aureus* in the model. Despite extended periods of concentrations of tazobactam less than the fixed concentration used in in vitro susceptibility testing (4 mg/liter), the bactericidal activity of piperacillin-tazobactam against TEM-3-producing *E. coli* was similar to that observed for piperacillin monotherapy against β-lactamase-negative *E. coli* J53.

The prolonged bactericidal effects of the combination

against β-lactamase-producing bacteria in the model might be explained by several mechanisms. Studies with tazobactam and other inhibitors indicate that its inactivation of β-lactamase is characterized by turnover rates of enzyme and inhibitor (4). Therefore, it is conceivable that several hours of an essentially β-lactamase-negative state may occur in bacteria following exposure to high levels of an inhibitor, until the production of enzyme by the persisting bacteria reaches levels sufficient to inactivate a significant proportion of the β-lactam. This period could result in a prolonged period of susceptibility of the bacteria to low concentrations of a β-lactam. Thorburn and Molesworth (27) reported a "post-β-lactamase inhibition effect" in which regrowth of amoxicillin-resistant bacteria was prevented for several hours by amoxicillin alone following a brief exposure to and then removal of amoxicillin plus clavulanic acid. Furthermore, Cavalieri et al. (5) reported augmentation of the antimicrobial effects of β-lactams by inhibitors in an animal model, even when the β-lactams were given up to 3.5 h after a single dose of the inhibitor. However, in the limited experiments conducted with the piperacillin-resistant strain of *E. coli* (J53-TEM-3), bacterial killing occurred only when tazobactam was present in the cultures, suggesting no post-β-lactamase inhibition effect of static concentrations of the combination of drugs against this strain. This result is consistent with previous observations with other β-lactam–β-lactamase inhibitor combinations (9).

As simulated in the model, β-lactamase inhibitor concentrations in vivo fall below the concentrations used for in vitro testing of combinations of β-lactam–β-lactamase inhibitor within 2 to 3 h after a dose. For tazobactam, concentrations in plasma exceed 4 mg/liter only up to 3 h after a dose. However, the AUC_{0-24} for tazobactam in the model was ~2 times greater than the amount of inhibitor that is available during incubation during susceptibility testing. This exposure profile was sufficient to enable bacterial killing by piperacillin in the model similar to that observed for the β-lactamase-negative isogenic strain. It appears that in vivo the AUC for an inhibitor is the important parameter for the activities of combinations with β-lactamase inhibitors against β-lactamase-producing bacteria. Thus, it is possible to prolong the dosage interval of a β-lactam–β-lactamase inhibitor combination for a finite period that is limited by the reaccumulation of the β-lactamase produced by persisting bacteria to levels that exceed the number of molecules of an inhibitor available to inactivate the β-lactamase.

The strategy of using prolonged dosage intervals of inhibitor combinations may not apply in all cases. Others have shown the rapid regrowth of bacteria in vitro after exposure to ticarcillin-clavulanic acid, removal of clavulanic acid, and resuspension in ticarcillin alone (9). Some strains with multiple copies of plasmids that hyperproduce β-lactamase or combinations of inhibitors with more labile β-lactams may not be amenable to prolonged inhibition of growth with extended dosage intervals.

The results of the present study show that the combination of piperacillin-tazobactam given as a daily exposure of 12 g of piperacillin and 1.5 g of tazobactam fractionated into dosage intervals of every 6 or 8 h resulted in similar degrees of bacterial killing under conditions that simulated the pharmacokinetics in humans in the in vitro model of infection used in the study. These results are concordant with human studies of 4 g of piperacillin plus 0.5 g of tazobactam every 8 h to patients with intra-abdominal infections, lower respiratory tract infections, and skin and soft-tissue infections (3, 20, 26) and suggest that further clinical evaluation of extended dosage intervals of this combination in humans is warranted.

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