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In Vitro Pharmacodynamics of Piperacillin, Piperacillin-Tazobactam, and Ciprofloxacin Alone and in Combination against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*

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The time-kill curve methodology was used to determine the pharmacodynamics of piperacillin, ciprofloxacin, piperacillin-tazobactam and the combinations piperacillin-ciprofloxacin and ciprofloxacin-piperacillin-tazobactam. Kill curve studies were performed for piperacillin, ciprofloxacin, and piperacillin-tazobactam at concentrations of 0.25 to 50 times the MICs for 13 strains of bacteria: four *Pseudomonas aeruginosa*, three *Enterobacter cloacae*, three *Klebsiella pneumoniae*, and three *Staphylococcus aureus* isolates (tazobactam concentrations of 0.5, 4, and 12 μg/ml). By using a sigmoid E_{max} model and nonlinear least squares regression, the 50% lethal concentrations and the maximum lethal rates of each agent were determined for each bacterial strain. For piperacillin-ciprofloxacin and ciprofloxacin-piperacillin-tazobactam, kill curve studies were performed with concentrations obtained by the fractional maximal effect method (R. C. Li, J. J. Schentag, and D. E. Nix, Antimicrob. Agents Chemother. 37:523–531, 1993) and from individual 50% lethal concentrations and maximum lethal rates. Ciprofloxacin-piperacillin-tazobactam was evaluated only against the four *P. aeruginosa* strains. Interactions between piperacillin and ciprofloxacin were generally additive. At physiologically relevant concentrations of piperacillin and ciprofloxacin, ciprofloxacin had the highest rates of killing against *K. pneumoniae*. Piperacillin-tazobactam (12 μg/ml) had the highest rate of killing against *E. cloacae*. Piperacillin-ciprofloxacin with relatively higher ciprofloxacin concentrations had the greatest killing rates against *S. aureus*. This combination had significantly higher killing rates than piperacillin ($P < 0.002$). For all the bacterial strains tested, killing rates by ciprofloxacin were significantly higher than those by piperacillin ($P < 0.001$). Piperacillin-tazobactam (4 and 12 μg/ml) had significantly higher killing rates than piperacillin alone ($P < 0.02$ and $P < 0.004$, respectively). The effect of the combination of piperacillin-ciprofloxacin, in which piperacillin concentrations were relatively higher, was not statistically different from that of piperacillin alone ($P \geq 0.71$). The combination of ciprofloxacin-piperacillin-tazobactam achieved greater killing than other combinations or monotherapies against *P. aeruginosa*. The reduction in the initial inoculum was 1 to 4 logs greater with ciprofloxacin-piperacillin-tazobactam at 4 and 12 μg/ml than with any other agent or combination of agents. On the basis of the additive effects prevalently demonstrated in the in vitro study, the combinations piperacillin-ciprofloxacin and piperacillin-tazobactam are rational therapeutic options. Greater killing of *P. aeruginosa* was demonstrated with ciprofloxacin-piperacillin-tazobactam. Since treatment failure of *P. aeruginosa* pneumonia is a significant problem, clinical studies are warranted.

Many studies have attempted to examine the efficacies of various combinations of antimicrobial agents by a wide range of susceptibility test methods including checkerboard synergy studies, serum bactericidal activity studies, and in vivo studies with animal models (2, 3, 7, 10). Unfortunately, divergent results have been obtained from these various studies, even in cases in which different studies evaluated the same antimicrobial combinations. In particular, combinations of fluoroquinolones and β-lactams have demonstrated interactions ranging from synergy to frank antagonism (1, 6, 12, 14). These discrepancies may be due in part to differences in susceptibility test methods. It is clear that better investigational methods are needed in order to reliably evaluate the effects of antimicrobial combinations.

We recently described the fractional maximal effect (FME) method for the evaluation of antimicrobial interactions (13) by the time kill-kinetic methodology, which allows for a continuous measure of concentration-effect relationships. In contrast, checkerboard synergy studies, which most often have been used to evaluate combinations of antibiotics in the past, use a broth dilution MIC technique which allows only an all-or-none response (growth or no growth) at one point in time rather than a continuous measurement of the effect. By initially assessing the concentration-effect relationship of the agents alone, the FME method mathematically predicts the effect of combinations of agents when a selected effect is targeted (i.e., 80% of the maximum effect), so that the measured effect can be characterized (i.e., antagonism, additivity, or synergism). One limitation of the method is failure to examine the full response surface. However, unlike checkerboard synergy studies which measure an all-or-none response, the FME method, which uses the kill-curve methodology, provides a continuous measure of effect.

The current study was performed in order to fully assess pharmacodynamic interactions such as maximum lethal rate (L_{max}) and the concentration required to achieve 50% of the maximal lethal rate (LC_{50}) which occur between piperacillin, piperacillin-tazobactam, and ciprofloxacin when tested against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. In particular, these inter-

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actions were compared by determining their potencies and lethal rates at clinically achievable concentrations in order to predict the optimal combination of these agents against the common nosocomial pathogens studied.

MATERIALS AND METHODS

Antimicrobial agents. The following analytical-grade standard powders were obtained from the indicated manufacturers: ciprofloxacin, Miles Inc., West Haven, Conn.; piperacillin and tazobactam, Lederle Laboratories, Pearl River, N.Y. Stock solutions were prepared according to the instructions provided by the manufacturers and were stored at −20°C. Only one freeze-thaw cycle was performed.

Inoculum preparation. Test organisms included *P. aeruginosa* (strains: ATCC 27853, 92-0158-2, 92-0110-2, and 93-0112), *S. aureus* (three strains: ATCC 25923, 93-0109-2, and 91-182-1189), *K. pneumoniae* (three strains: ATCC 13883, 92-0207, and 91-263-0505), and *E. cloacae* (three strains: ATCC 23355, 89-142, and 92-0190). Single bacterial colonies from an overnight agar plate were touched with a sterile loop and added to a tube containing cation-adjusted Mueller-Hinton broth (CA-MHB; Mueller-Hinton broth supplemented with Ca^{2+} at 20 to 25 mg/liter and Mg^{2+} at 10 to 12.5 mg/liter). The tubes were incubated at 35°C in order to achieve bacterial logarithmic growth and a density equal to that of a no. 0.5 McFarland standard. Dilution of the inoculum was achieved in CA-MHB. The final inoculum size was verified each time by colony counting by a spread plate technique.

MIC determination. MICs were determined with CA-MHB by using the guidelines of the National Committee for Clinical Laboratory Standards (15). The final inoculum was prepared from logarithmic-phase bacteria and was approximately 5×10^5 CFU/ml. This inoculum was added to 0.1 ml of CA-MHB containing serial twofold dilutions of the antimicrobial agents. Microdilution trays were incubated at 35°C for approximately 18 to 24 h. The well containing the lowest concentration of antibiotic that prevented visible growth was defined as the MIC.

Single-agent studies. Ciprofloxacin and piperacillin kill curve studies were performed at concentrations of 0.25, 0.5, 1, 1.5, 2, 4, 25, 30, 35, and 50 times the MIC for each test organism. The concentration/MIC ratios were chosen by using optimal sampling strategy and ADAPT II (Biomedical Simulations Resource, University of Southern California, Los Angeles). A preliminary study was performed in order to obtain a concentration-effect relationship for each individual agent at 0.5, 1, 5, 10, 20, 25, 30, 35, 40, 45, and 50 times the MIC. Optimal sampling looks for regions in the effect curve which show the greatest amount of variability not explained by assay variability. In this manner, the multiples of the MIC which would provide the most information with the least amount of sampling were selected. Piperacillin-tazobactam kill curve studies also were performed at piperacillin concentrations ranging from 1/4 to 50 times the MIC of piperacillin with 0.5, 4, or 12 μg of tazobactam per ml. We chose 4 μg/ml since it is the concentration used for standard susceptibility testing. Two additional concentrations, one higher and one lower than the standard, were chosen in order to determine whether a concentration-effect relationship exists for the addition of tazobactam to piperacillin.

Twenty-five milligrams of activated charcoal was used in order to remove ciprofloxacin prior to plating. This method was first validated to ensure antibiotic removal without loss of bacterial viability. Growth curves were performed over a 3-h incubation period with ciprofloxacin concentrations of 5 to 50 μg/ml, and 25 mg of activated charcoal was added to each sample. Counts of CFU per milliliter at 3 h were no lower for samples containing ciprofloxacin than for the control samples, indicating good removal of antimicrobial activity. One hundred microliters of β-lactamase (500,000 IU/ml) (Penase; Difco Laboratories, Detroit, Mich.) was added to the samples in order to eliminate the activity of piperacillin.

Inocula were prepared from overnight cultures of each organism in CA-MHB. The final inoculum was prepared by taking 1 ml of the prepared inoculum at a no. 0.5 McFarland standard and adding it to 9 ml of CA-MHB. One hundred microliters of the inoculum was added to 0.9 ml of the antibiotic solution(s) at the appropriate concentration in CA-MHB. This resulted in a final inoculum of approximately 10^6 CFU/ml. Each of the prepared inoculum-antibiotic combinations was incubated at 35°C along with control and control with antibiotic–charcoal–β-lactamase combinations. Samples were removed for determination of bacterial colony counts at 0 and 4 h of incubation.

One hundred-microliter samples containing ciprofloxacin were added to 900 μl of normal saline with 25 mg of activated charcoal. Further dilutions were performed by removing 100 μl of this solution and diluting it in 900 μl of normal saline. Three different dilutions for each initial sample were plated by the pour plate method with Mueller-Hinton agar. Plates were incubated for 18 to 24 h at 35°C, and colony counts were determined. The rejection value, based on the initial inoculum, was <30 colonies (16).

One hundred-microliter samples containing piperacillin were added to 800 μl of normal saline and 100 μl of β-lactamase. Further dilutions and plating were performed by the method described earlier.

For the piperacillin-tazobactam samples, serial dilution in normal saline was performed. The samples were then filtered through 0.45-μm-pore-size cellulose acetate filters (Whatman Ltd., Maidstone, England), and the filters were rinsed with 20-ml portions of normal saline to remove antibiotic. Two milliliters of CA-MHB was added to a sterile absorbent disk in a petri dish, and the filter was placed on the disk. All samples were incubated for 18 to 24 h at 35°C prior to obtaining the colony counts.

Growth and kill rates were calculated by using the following equations: kill rate constant = growth rate constant − apparent kill rate constant, growth rate constant = [ln CFU (4 h)$_{control}$ − ln CFU (initial)]/time interval (4 h), and apparent kill rate constant = [ln CFU (4 h)$_{tx}$ − ln CFU (initial)]/time interval (4 h), where *tx* is treatment (ciprofloxacin, piperacillin, or piperacillin-tazobactam).

Combination studies. Concentration effect curves (as concentration/MIC versus kill rate) from the single-agent studies were fit using an E_{max} model (maximal attainable effect model), sigmoid E_{max} model (E_{max} model utilizing Hill's constant to define the steepness of the curve), and C_{mec} model (sigmoid E_{max} model where a minimum effective concentration is required for effect to be seen) and nonlinear regression (PCNONLIN nonlinear estimation program, VO3.0). The best fit was consistently provided by the sigmoid E_{max} model on the basis of Akaike's Information Criterion. The parameters L_{max} and LC_{50} were estimated for the model and were used to describe the concentration-effect curve. The FME method uses the individual concentration-effect curves to determine the concentrations of pairs of drugs that can be used to assess synergy on an isobologram. A total FME value of 0.8 was used in these calculations. Both single-agent and combination studies were performed on six separate occasions on two organisms (*S. aureus* 93-0109-2 and *E. cloacae* 89–142) in order to examine the reproducibility of the method.

One hundred-microliter samples containing both ciprofloxacin and piperacillin were added to 800 μl of normal saline with 100 μl of β-lactamase and 25 mg of activated charcoal. Further dilutions and plating were performed by the methods described earlier. For the ciprofloxacin-piperacillin-tazobactam samples, the antibiotics were eliminated and the samples were prepared in the same manner described for the piperacillin-tazobactam samples (see section on single-agent studies).

Statistical analysis. Two-way analysis of variance was used to test whether a significant difference existed between the different species of bacteria and different treatments with respect to LC_{50} and L_{max}. The treatments tested included ciprofloxacin alone, piperacillin alone, and piperacillin-tazobactam with 0.5, 4, and 12 μg of tazobactam per ml.

In order to compare single-agent versus combination treatments, treatments were evaluated for the cases in which ciprofloxacin concentrations were approaching but were not greater than 2 μg/ml and piperacillin concentrations were approaching but were not greater than 32 μg/ml. These represent concentrations that are achievable after standard dosing of each of the agents. For the ciprofloxacin-piperacillin studies, two cases were evaluated for each study. The first case contained ciprofloxacin at a concentration approaching 2 μg/ml and piperacillin at a relatively low concentration. The second case contained piperacillin at a concentration approaching 32 μg/ml and ciprofloxacin at a relatively low concentration. Analysis of variance was performed in order to test for a significant difference in killing rates between treatments and between bacterial strains. In the ciprofloxacin-piperacillin-tazobactam studies, comparisons were made for the four *P. aeruginosa* strains only.

RESULTS

Bacterial MICs. MICs were determined on three separate occasions and were reproducible to within ±1 dilution. The piperacillin, ciprofloxacin, and piperacillin-tazobactam MICs for each organism are given in Table 1. The addition of tazobactam at 4.0 and 12.0 μg/ml to piperacillin resulted in lower MICs for *K. pneumoniae* and *S. aureus* isolates, but it had no effect on *P. aeruginosa* or *E. cloacae* strains.

Monotherapy studies: concentration versus killing rate. Ciprofloxacin achieved a greater maximal rate of killing against each of the bacterial strains compared with the rate for piperacillin. In addition, concentration-dependent killing was more evident with ciprofloxacin than with piperacillin. However, for the *S. aureus* strains, maximal killing rates were demonstrated at about four times the MIC of ciprofloxacin and two times the MIC of piperacillin. For the gram-negative organisms, ciprofloxacin demonstrated concentration-dependent killing at concentrations up to 50 times the MIC. For piperacillin, an increase in the killing rate against the gram-negative organisms was seen at concentrations up to 20 times the MIC.

Figures 1 and 2 display the relationship between the killing rate and the antibiotic concentration/MIC ratio for *S. aureus* 93-0109-2 and *E. cloacae* 89-142. Each point represents the mean value for the six studies with each bacterial strain and antimicrobial agent. The bars at each point are the 95% confidence intervals around the mean value. For ciprofloxacin and *S. aureus* 93-0109-2, concentration-dependent killing was ex-

TABLE 1. Antimicrobial susceptibilities for ciprofloxacin, piperacillin, and piperacillin-tazobactam

Bacterial strain	MIC (μg/ml)[a]				
	Cipro	Pip	P/T-0.5	P/T-4	P/T-12
P. aeruginosa					
ATCC 27853	0.5	12	6	3	6
92-0158-2	0.5	6	12	12	6
92-0110-2	0.125	64	96	96	96
93-0112	0.125	8	6	6	6
E. cloacae					
ATCC 23355	0.016	3	12	3	3
89-142	0.03	3	24	6	6
92-0190	0.125	48	96	96	96
K. pneumoniae					
ATCC 13883	0.016	6	12	3	3
92-0207	0.0625	48	24	6	6
91-263-0505	0.05	24	12	3	3
S. aureus					
ATCC 25923	0.5	12	12	3	3
91-182-1189	0.25	48	24	6	6
93-0109-2	0.25	48	12	1.5	1.5

[a] Cipro, ciprofloxacin; Pip, piperacillin; P/T-0.5, P/T-4, and P/T-12, piperacillin-tazobactam with tazobactam at 0.5, 4.0 and 12.0 μg/ml, respectively.

hibited at concentrations up to approximately two times the MIC, and no further increases in the killing rate were seen at higher concentrations. We chose the concentration/MIC ratio associated with 90% of the L_{max} as the value identified with the extent of concentration-dependent killing. For instance, in the previous example, concentration-dependent killing was seen up to a concentration/MIC ratio of 2:1, at which 90% of the L_{max} was achieved.

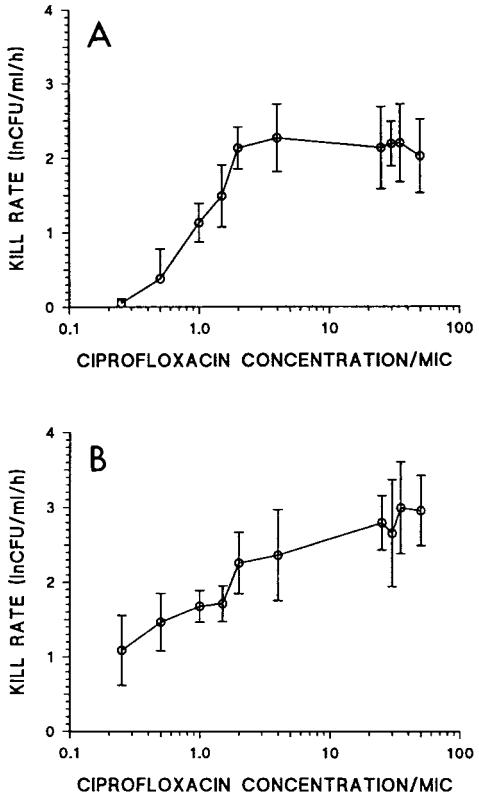

FIG. 1. Mean killing rates with 95% confidence intervals for ciprofloxacin at 0.25 to 50 times the MIC for *S. aureus* 93-0109-2 (A) and *E. cloacae* 89-142 (B).

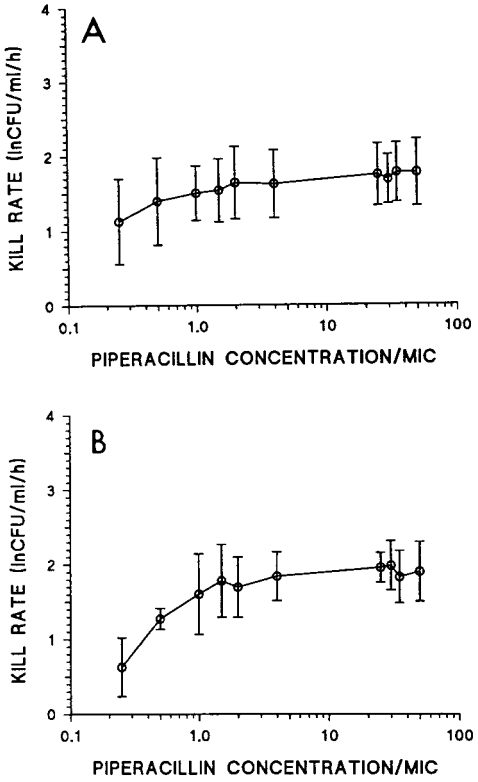

FIG. 2. Mean killing rates with 95% confidence intervals for piperacillin at 0.25 to 50 times the MIC for *S. aureus* 93-0109-2 (A) and *E. cloacae* 89-142 (B).

Figure 1A shows the relationship between killing rate and ciprofloxacin concentration/MIC ratio for *S. aureus* 93-D109-2. Concentration-dependent killing was seen at concentrations up to 4 times the MIC. Figure 1B shows the relationship between the killing rate and ciprofloxacin concentration/MIC ratio for *E. cloacae* 89-142. Concentration-dependent killing was seen at concentrations up to 20 to 40 times the MIC. Figure 2A shows the relationship between the killing rate and the piperacillin concentration/MIC ratio for *S. aureus* 93-0109-2. Although some concentration-dependent killing was seen, the maximum effect was noted at 0.25 to 2 times the MIC. Figure 2B shows the relationship between the killing rate and piperacillin concentration for *E. cloacae* 89-142. A similar concentration-dependent effect was evident for piperacillin against this organism, against which the maximum killing rate was apparent at 0.25 to 4 times the MIC.

Greater variability was noted in the LC_{50}s of piperacillin than in those of ciprofloxacin in the six replicate studies with *S. aureus* 93-0109-2 (coefficients of variation, 169.8 and 32.6%, respectively). Less variability was seen in the L_{max}s for each of these agents with this organism (coefficients of variation, 19.5 and 24.2%, respectively). For the studies with *E. cloacae* 89-142, LC_{50}s of piperacillin and ciprofloxacin had similar variabilities (coefficients of variation, 33.1 and 46.2%, respectively). Once again, less variability was noted for the L_{max}s (coefficients of variation, 7.5 and 15.7, respectively).

The L_{max}s and LC_{50}s of piperacillin and piperacillin-tazobactam are given in Table 2. In comparison with piperacillin

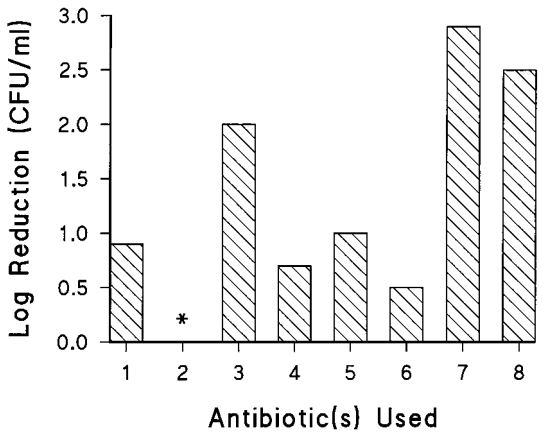

FIG. 3. Bacterial killing of *P. aeruginosa* ATCC 27853 by antimicrobial agents. 1, ciprofloxacin at 1.0 μg/ml; 2, piperacillin at 3.0 μg/ml; 3, ciprofloxacin-piperacillin at 1.3 and 1.5 μg/ml, respectively; 4, piperacillin-tazobactam at 3.0 and 0.5 μg/ml, respectively; 5, piperacillin-tazobactam at 3.0 and 4.0 μg/ml, respectively; 6, piperacillin-tazobactam at 3.0 and 12.0 μg/ml, respectively; 7, ciprofloxacin-piperacillin-tazobactam at 0.7, 0.01, and 4.0 μg/ml, respectively; 8, ciprofloxacin-piperacillin-tazobactam at 0.8, 0.8, and 12 μg/ml, respectively. *, no activity was noted for piperacillin alone at 3.0 μg/ml.

alone, the addition of tazobactam at 0.5, 4, and 12 μg/ml significantly enhanced potency, as evidenced by the lower LC_{50}s. This enhanced potency was not concentration dependent, because differences in effect were not significant between different tazobactam concentrations. Significantly higher L_{max}s were achieved with the addition of 4 or 12 μg of tazobactam per ml. This difference was not seen when 0.5 μg of tazobactam per ml was used. No greater effect was achieved, however, with 12 μg/ml than with 4 μg/ml.

Combinations of ciprofloxacin, piperacillin, and tazobactam: killing rate studies. The FME plots for the piperacillin-ciprofloxacin interaction studies for *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *E. cloacae* strains are given in Fig. 4. The interaction ratios (*R*) are plotted versus the ciprofloxacin-to-piperacillin concentration ratios. An *R* value of 1 is equal to additivity (13). An *R* value of less than 0.5 describes a synergistic interaction, while an *R* value of greater than 2 describes antagonism. The values used to describe interaction (i.e., synergy or antagonism) were chosen on the basis of the results of the six replicate studies described earlier, since the variability seen was accommodated through either doubling or halving the interaction ratio. By these definitions, the interactions between piperacillin and ciprofloxacin were additive for the four *P. aeruginosa* strains. Antagonism was seen at low ciprofloxacin/piperacillin concentration ratios for one of the three *E. cloacae* strains. A second *E. cloacae* strain showed antagonism at the higher ciprofloxacin-to-piperacillin concentration ratios. Interactions were additive for two of the three *K. pneumoniae* strains, while antagonism was displayed for the third strain at the lower ciprofloxacin-to-piperacillin concentration ratios. Additivity was noted for two of the three *S. aureus* strains, while antagonism was displayed for the third strain, most notably at low ciprofloxacin-to-piperacillin concentration ratios.

For the studies which assessed the reproducibility of the FME method, antagonism was noted for *E. cloacae* at the lower ciprofloxacin-to-piperacillin concentration ratios. The trend was toward additivity at the higher ciprofloxacin-to-piperacillin concentration ratios. For *S. aureus*, additivity was apparent at all ciprofloxacin-to-piperacillin concentration ratios. In each case, the results were highly reproducible. Although the trends were similar for each repeat for the *E. cloacae* strains, there was greater variability in the results than those noted for the *S. aureus* strains.

The combination of ciprofloxacin-piperacillin-tazobactam achieved greater rates of killing against *P. aeruginosa* than did the other monotherapies or combinations. In all cases 1- to 4-log greater reductions in the initial inoculum were demonstrated with ciprofloxacin-piperacillin-tazobactam with tazobactam concentrations of 4 and 12 μg/ml.

Figure 3 compares the logarithmic reduction of a strain of *P. aeruginosa* with seven different agents or combinations of agents. Since it was necessary to use very low concentrations of the three agents for the ciprofloxacin-piperacillin-tazobactam combination studies, treatments were compared across all agents at relatively low concentrations. The combinations of ciprofloxacin-piperacillin-tazobactam resulted in the greatest logarithmic reduction in the initial inoculum.

Statistical analysis. The differences in killing rates between these treatments were statistically significant for piperacillin and piperacillin-tazobactam with tazobactam concentrations of 12 μg/ml ($P < 0.03$). In these studies the piperacillin-to-tazobactam concentration ratios ranged from 0.0625:1 to 267:1. A piperacillin-to-tazobactam concentration ratio of 8:1 is typically assessed for in vitro susceptibility testing. Our piperacillin concentration ratios were chosen by using multiples of the MIC. Maximal killing rates were similar at similar multiples of piperacillin MICs with tazobactam concentrations of 12 μg/ml, indicating that a sufficient amount of β-lactamase inhibitor was present even at the higher piperacillin concentrations.

The difference in killing rates between piperacillin and piperacillin-tazobactam at the lower tazobactam concentrations did not reach statistical significance. The differences in the LC_{50}s were statistically significant, with $P < 0.05$ for each of the three tazobactam concentrations compared with piperacillin alone. Ciprofloxacin monotherapy displayed lower LC_{50}s than those of piperacillin monotherapy or therapy with any of the piperacillin-tazobactam concentrations. These differences

TABLE 2. L_{max} and LC_{50}s of piperacillin and piperacillin-tazobactam[a]

Bacterial strain	P/T-0.5		P/T-4		P/T-12		Piperacillin	
	L_{max}	LC_{50}	L_{max}	LC_{50}	L_{max}	LC_{50}	L_{max}	LC_{50}
P. aeruginosa								
92-0158-2	1.860	0.524	2.663	0.999	2.340	0.506	0.797	0.491
ATCC 27853	2.900	0.264	2.415	0.148	2.548	0.291	1.748	0.214
92-0110-2	1.685	0.101	1.605	0.122	2.337	0.126	1.983	1.929
93-0112	2.389	0.285	2.448	0.298	2.445	0.345	1.549	0.496
E. cloacae								
92-0190	2.408	0.331	2.526	0.305	2.716	0.042	2.266	0.562
ATCC 23355	1.387	0.009	1.506	0.011	1.538	0.005	0.819	1.800
89-142	2.244	0.191	2.354	0.294	2.572	0.240	2.043	0.142
K. pneumoniae								
ATCC 13883	2.452	0.121	3.067	0.334	2.510	0.271	3.609	0.856
91-263-0505	2.749	0.046	2.734	0.001	3.196	0.037	2.248	0.578
92-0207	1.945	0.086	1.960	0.027	2.300	0.007	2.698	1.050
S. aureus								
ATCC 25923	1.757	0.174	2.132	0.131	1.915	0.127	1.190	0.041
91-182-1189	2.196	0.028	1.890	0.138	1.752	0.049	1.551	0.385
93-0109-2	1.644	0.073	1.485	0.058	2.425	0.035	1.449	0.067

[a] P/T-0.5, P/T-4, and P/T-12, piperacillin-tazobactam with tazobactam at concentrations of 0.5, 4.0, and 12.0 μg/ml, respectively. Piperacillin was used at 0.25 to 50 times its MIC. L_{max} is expressed in units of CFU per milliliter per hour. LC_{50} is a concentration-to-MIC ratio.

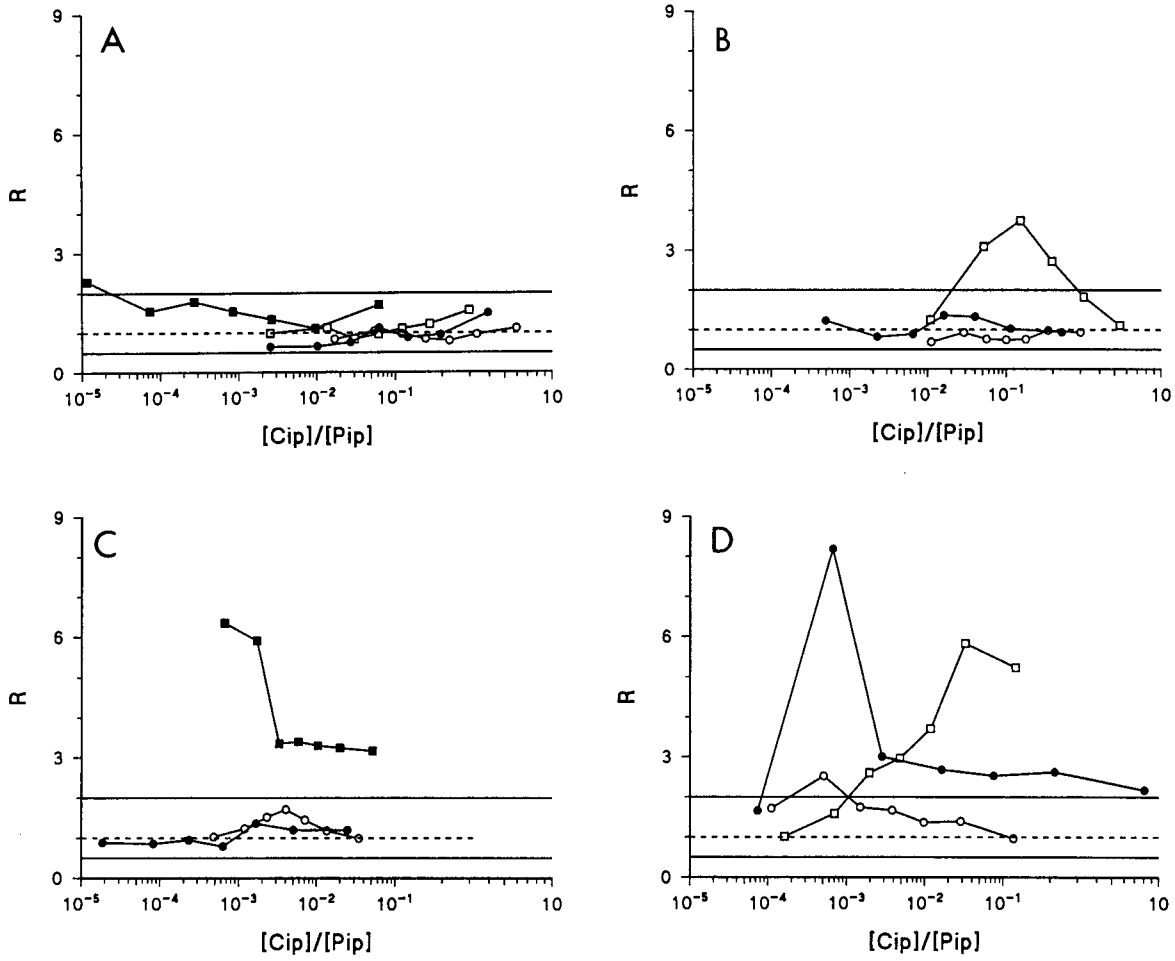

FIG. 4. FME plots for *P. aeruginosa* 92-0110-2 (■), ATCC 27853 (□), 93-0112 (●), and 92-0158-2 (○) (A); *S. aureus* ATCC 25923 (□), 91-182-1189 (●), and 93-0109-2 (○) (B); *K. pneumoniae* ATCC 13883 (■), 92-0207 (●), and 93-263-0505 (○) (C) and *E. cloacae* ATCC 23355 (□), 89-142 (●), and 92-0190 (○) (D). The dashed line ($R = 1.0$) represents the line of additivity. The solid lines flank the region of additivity. [Cip], ciprofloxacin concentration; [Pip], piperacillin concentration.

in LC_{50}s were significant ($P < 0.03$) in all cases. The killing rates of ciprofloxacin were greater than those of piperacillin and piperacillin-tazobactam at tazobactam concentrations of 0.5 and 4 μg/ml. These differences were significant for piperacillin and piperacillin-tazobactam at tazobactam concentrations of 0.5 μg/ml ($P < 0.04$) versus ciprofloxacin. A statistical difference in LC_{50}s and L_{max}s between bacterial species or bacterial strains was not achieved.

In studies in which treatments were compared at ciprofloxacin concentrations approaching 2 μg/ml and piperacillin concentrations approaching 32 μg/ml, a statistical difference in killing rates was noted between ciprofloxacin and both piperacillin and piperacillin-tazobactam with tazobactam concentrations of 0.5 μg/ml ($P < 0.001$ and $P < 0.04$, respectively). For the piperacillin-ciprofloxacin combinations, the studies with relatively higher ciprofloxacin concentrations achieved statistically greater killing rates than piperacillin ($P < 0.001$). Piperacillin-tazobactam at tazobactam concentrations of 0.5, 4, and 12 μg/ml achieved statistically higher killing rates than piperacillin ($P < 0.04$) in all three studies. Differences in killing rates between bacterial strains were also significant ($P < 0.002$). Killing rates against *P. aeruginosa* were significantly greater for ciprofloxacin-piperacillin-tazobactam than for piperacillin when tazobactam concentrations were 4 or 12 μg/ml ($P = 0.002$ and $P = 0.0004$, respectively).

DISCUSSION

Over the past several decades, a considerable number of very potent antimicrobial agents have been developed. In spite of these new therapeutic options, the successful treatment of nosocomial infections remains a serious problem. Bacteria that demonstrate in vitro susceptibility to the agent chosen may be able to develop resistance during treatment (5, 9, 11) because of the selection of a more resistant subpopulation of bacteria for which MICs are much greater than those for the main bacterial population (3). Toxicity considerations may limit our ability to increase doses of available agents in order to achieve concentrations in serum which might adequately treat these infections. Combinations of antimicrobial agents that exhibit synergistic or additive activity over the individual agents could improve the outcomes of difficult-to-treat infections.

Interactions between fluoroquinolones and other classes of antimicrobial agents have been assessed by various methodologies. Only sporadic occurrences of synergy have been reported for ciprofloxacin and β-lactam combinations against members of the family *Enterobacteriaceae* (4, 6). Synergy has been demonstrated between ciprofloxacin and azlocillin against both *P. aeruginosa* (8) and *S. aureus* (6). There is a low likelihood of antagonistic interactions between fluoroquinolones and β-lactams (8).

We found the combination of piperacillin-ciprofloxacin to

have additive activity against each of the *P. aeruginosa* strains. This is an important observation since monotherapy for *P. aeruginosa* may result in clinical failure, and an alternative to aminoglycoside–β-lactam combinations may be desirable in some patients. For the *E. cloacae*, *K. pneumoniae*, and *S. aureus* strains, the piperacillin-ciprofloxacin combination was generally additive. When antagonism was encountered, it was seen at the lower ciprofloxacin-piperacillin concentration ratios. Additivity was demonstrated in these same organisms studied at the higher ciprofloxacin-piperacillin concentration ratios, as might be expected when an antibiotic with concentration-dependent killing is paired with a antimicrobial agent with concentration-independent killing. In addition to species differences in interactions, differences were noted between strains in some cases, making it difficult to characterize the nature of combination effects for a particular species.

Two of the 13 organisms were selected in order to test the reproducibility of the FME method. The initial studies with each of these organisms were repeated five additional times. In each case, the method proved to be reproducible. The combination was additive for the *S. aureus* strain. For the *E. cloacae* strain, the combination was reproducibly antagonistic at the lower ciprofloxacin/piperacillin concentration ratios and additive at the higher ratios.

Each of the antibiotics in the piperacillin-tazobactam regimens was evaluated as an individual agent rather than as a combination. We felt that this was an appropriate approach since tazobactam alone has little antimicrobial activity. This allowed each piperacillin-tazobactam dosage to be evaluated for its activity relative to that of piperacillin. Tazobactam enhanced both the potency and the rate of killing of piperacillin. The LC_{50} is a measure of relative potencies between agents, with a lower LC_{50} representing an increase in potency. The increased potency with the addition of tazobactam was anticipated, since hydrolysis of piperacillin by β-lactamases would be expected to decrease the active concentration of this drug. The mechanism for the increase in the rate of killing with piperacillin-tazobactam is not as clear. It is possible that the higher active concentrations achieved with piperacillin-tazobactam were greater than the MIC for a greater percentage of organisms in the initial inoculum, enhancing the observed rate of killing (through a greater reduction in the numbers of CFU per milliliter at 4 h of incubation).

The combination of ciprofloxacin-piperacillin-tazobactam achieved the greatest rates of killing against *P. aeruginosa* compared with that of either agent alone or the combination of piperacillin-ciprofloxacin. The inducible β-lactamases of *P. aeruginosa* are not generally inhibited by tazobactam. Yet, greater activity against four strains of *P. aeruginosa* was achieved with the addition of tazobactam to the piperacillin-tazobactam concentration. A mechanism for an added effect with the addition of tazobactam remains to be examined.

By comparing monotherapies and combination therapies at physiologically relevant concentrations, we are better able to predict whether the results that we achieved in vitro might have clinical significance. Although these results obtained from an in vitro study cannot be directly extrapolated to the clinical situation, the clinical efficacy of a given agent is generally reflected by that agent's in vitro activity.

It is easiest to demonstrate a synergistic relationship with low concentrations of two agents when using time-kill curve methods. In contrast, high concentrations of two agents are more apt to demonstrate antagonism if a maximum rate of killing can be achieved by a single agent at the concentration tested. These limitations must be considered when evaluating results of synergy studies which use this methodology.

Antagonism is an effect achieved with the combination of two agents which is less than the combined effects of the two agents used individually. Although a synergistic effect is most desirable, an antagonistic effect may still achieve results superior to those achieved with monotherapy. For instance, an antagonistic combination of agents may be useful if the addition of the second agent can achieve an effect greater than that of either agent used individually. Patients with difficult-to-treat infections may benefit from combination therapy even if the agents used have failed to show an additive or synergistic relationship.

In summary, the combination therapies assessed in the present study were found to be generally additive. However, enhanced bactericidal activity was seen with combination therapy, and this difference in activity was statistically significant for several of the combinations. Further evaluation in human clinical trials will be necessary in order to determine microbiologic and clinical responses in an infected patient population.

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