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Pharmacodynamics of Once-Daily Amikacin in Various Combinations with Cefepime, Aztreonam, and Ceftazidime against *Pseudomonas aeruginosa* in an In Vitro Infection Model

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The pharmacodynamics of once-daily amikacin administered as monotherapy and in combination with aztreonam, ceftazidime, and cefepime against *Pseudomonas aeruginosa* ATCC 27853 and clinical isolate 16690 (moderately susceptible to ceftazidime) were investigated with an in vitro model of infection over a 24-h period. Monotherapy with aztreonam, ceftazidime, and cefepime and combinations of aztreonam with cefepime or ceftazidime were also studied. MICs and MBCs were determined for viable organisms at 24 h to test for the development of resistance. Once-daily amikacin demonstrated killing activity over the initial 8 h superior to those of all other drugs administered as monotherapy against both strains tested ($P < 0.01$). Regrowth by 24 h was greatest for the amikacin regimen ($P < 0.01$) but was apparent for all monotherapy regimens against both strains. No changes in susceptibilities were observed. All combination therapies containing once-daily amikacin achieved 99.9% reductions in $\log_{10}$ CFU/ml by 2.0 h against both strains, with no regrowth of organisms at 24 h. Aztreonam-cefepime and -ceftazidime combinations required approximately 5 to 6 h to achieve a 99.9% reduction in $\log_{10}$ CFU/ml. Although there was no difference in time to the 99.9% kill between the aztreonam-cefepime and -ceftazidime regimens against either strain, the killing activity of these combinations was significantly less than those of regimens containing once-daily amikacin ($P < 0.01$). Minor differences in the initial susceptibilities of β-lactams and the monobactam aztreonam against *P. aeruginosa* may not be important for therapeutic outcomes when used in combination with single-daily aminoglycoside therapy.

Despite recent advances in the development of new antimicrobial agents, aminoglycosides remain an important class of antibiotics for the treatment of infections caused by gram-negative bacilli. Aminoglycosides possess several established pharmacodynamic features which contribute to their bactericidal activities, including concentration-dependent killing activity, a postantibiotic effect, synergy with β-lactam agents, and the absence of a significant inoculum effect (4, 15). Studies with both animals and patients have supported the efficacy of once-daily, large doses of aminoglycosides for serious gram-negative infections, including appendicitis and other intra-abdominal infections, bacteremia, and *Pseudomonas aeruginosa* pneumonia (14, 16, 18, 21, 25, 28, 32).

It has been speculated previously that the role of aminoglycoside therapy in infectious diseases may change if single-daily doses are adopted (16, 30). In this case, the aminoglycoside would offer synergistic killing activity and would potentially curtail the emergence of resistance, while the monobactam or β-lactam would be primarily responsible for maintaining bactericidal activity as aminoglycoside levels decline below detectable limits and beyond the theoretical limits of the postantibiotic effect. There is also some evidence that supports the use of double β-lactam therapy for gram-negative infections (5, 6, 15). Clinical trials comparing combinations of an aminoglycoside–β-lactam with double β-lactam regimens have failed to show differences in clinical response rates (5, 6). These novel combinations require evaluation of their synergistic potential against infections caused by organisms with inherently lower sensitivities (e.g., *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacter* spp.). This is of particular concern in the treatment of infections in immunocompromised patients, in which efficacy is highly dependent upon the killing activities of antibiotics.

Serious pseudomonal infections are commonly treated with the combination of an aminoglycoside and an antipseudomonal β-lactam such as ceftazidime or aztreonam. Cefepime is a new, broad-spectrum parenteral cephalosporin with a gram-negative spectrum of activity similar to that of ceftazidime (2, 3). The purpose of this investigation was to determine whether the bactericidal activities of once-daily amikacin in combination with cefepime, aztreonam, or ceftazidime were similar against two strains of *P. aeruginosa* with differing patterns of susceptibility. Aztreonam in combination with cefepime or ceftazidime, herein referred to as a double β-lactam combination, was also studied in order to compare its bactericidal activity against those of once-daily amikacin combinations.

MATERIALS AND METHODS

Bacterial strains. The following two strains of *P. aeruginosa* were studied: ATCC 27853 and a clinical isolate, 16690. MICs and MBCs were determined by a microdilution technique following National Committee for Clinical Laboratory Standards guidelines with a starting inoculum of 5×10^5 CFU/ml (Table 1) (20). Sensitivities were determined both before exposure with the model and for viable organisms at 24 h. Both strains were equally sensitive to amikacin,

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TABLE 1. Susceptibilities of *P. aeruginosa* strains to antimicrobial agents[a]

Organism at (h):	MIC/MBC (μg/ml)								
	AK	AZ	CP	CF	AK-AZ	AK-CP	AK-CF	AZ-CP	AZ-CF
ATCC 27853									
0	2/4	4/8	1/2	2/4					
24	2/4	4/16	1/2	1/4	2/4-4/8	ND	ND	4/8-2/4	4/8-2/4
FIC/FBC 16690					0.5/2	0.38/1.5	0.25/2	1.5/1.5	2.5/5
0	2/4	8/16	4/16	16/32					
24	2/4	8/16	2/8	16/32	ND	ND	ND	8/16-4/8	4/8-8/16
FIC-FBC					0.31/1.25	0.31/0.31	0.31/3	0.5/1	0.75/3

[a] AK, amikacin; AZ, aztreonam; CP, cefepime; CF, ceftazidime; ND, not determined; FIC/FBC, fractional inhibitory concentration/fractional bactericidal concentration.

aztreonam, and cefepime; ATCC 27853 was sensitive and strain 16690 was moderately susceptible to ceftazidime.

Antibiotics. Aztreonam (analytical powder [batch no. 05314-38275] and powder for injection [lot no. 1E46121]) and cefepime (analytical powder [batch no. D10029] and powder for injection [batch no. MG9124]) were supplied by Bristol-Myers Squibb, Princeton, N.J. Amikacin (lot G1J05A; Bristol-Myers Squibb, Evansville, Ind.) and ceftazidime (analytical powder [batch no. UCRZ2125; Glaxo Group Research, Ware, Herts, United Kingdom] and powder for injection [B85331BA; Glaxo, Research Triangle Park, N.C.]) were also used. Stock solutions were prepared in appropriate amounts of distilled, deionized, sterile water.

Medium. Mueller-Hinton broth (BBL, Becton Dickinson, Cockeysville, Md.) supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter) was used for all susceptibility and model experiments.

In vitro model. A two-compartment in vitro infection model which allows for the simulation of human pharmacokinetics in the presence of bacteria was utilized as previously described (10). Drugs were bolused (1.0 ml) into the central compartment represented by a 275-ml-volume specially prepared glass container with a peripheral compartment consisting of a 7-ml-volume hollow glass T tube fitted on each end with an inert, 0.2-μm-pore-size polycarbonate membrane. This membrane allows for the passage of drugs but prohibits the migration of bacteria. Clearance of the agents from the central compartment was simulated by adding drug-free medium with a peristaltic pump set to achieve 2-h half-lives for all drug regimens. The model was maintained in a water bath at 37°C. All experiments were performed in duplicate. The following targeted mean peak concentrations for the various antibiotics were based on clinically achievable concentrations in patients being treated for serious infections: amikacin (15 mg/kg of body weight) (80 μg/ml) and 2-g doses of cefepime (140 μg/ml), aztreonam (100 μg/ml), and ceftazidime (160 μg/ml) (3, 17, 19, 26, 29). A second dose of β-lactam was administered at 8 h. Each drug regimen was administered as monotherapy and in combination with aztreonam or once-daily amikacin. Bacterial inocula were prepared from an overnight growth of 3 to 5 colonies suspended in 3 ml of Mueller-Hinton broth supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter) incubated at 37°C. Overnight cultures were diluted 1:3,000 and reincubated for 1 h at 37°C. One milliliter of this dilution was added to the peripheral compartment and allowed to equilibrate for 1 h to ensure exponential growth and to reach a starting inoculum of $\sim 10^6$ CFU/ml.

Pharmacodynamic analysis. Samples (0.1 ml) were removed from the peripheral compartment at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after administration of the initial dose to determine CFU of bacteria per ml. Suitable 10-fold dilutions with cold 0.9% sodium chloride were made, and 20 μl was plated on tryptic soy agar in triplicate. The plates were allowed to incubate at 37°C for 18 to 24 h, and the colonies were counted. At time points at which bacterial counts were expected to be below the limits of detection, 100-μl samples were placed in 10 ml of cold 0.9% sodium chloride and filtered by a Millipore system (0.45-μm-pore-size filter). Filters were placed aseptically on tryptic soy agar and incubated for 18 to 24 h, and the colonies were counted. The theoretical limit of detection for this method is 10 CFU/ml. Experiments in our laboratory have demonstrated a reliable limit of detection of 100 CFU/ml ($n = 20$; coefficient of variation, 10.2%). Antibiotic carryover experiments using simulated drug concentrations from the model did not inhibit the growth of a known inoculum of *P. aeruginosa*. Kill curves were constructed by plotting the $\log_{10}$ CFU/ml versus time. The time to achieve a 99.9% reduction in $\log_{10}$ CFU/ml was determined visually from the kill curves.

Pharmacokinetic analysis. Samples (0.1 ml) were removed from the central and peripheral compartments at 0.5, 3, 5, and 24 h to determine drug concentrations. Half-lives were determined from the slope of the concentration (central compartment) versus the time curve.

Antibiotic assay. Amikacin concentrations were determined by fluorescence polarization immunoassay (TDx; Abbott Laboratories, Irving, Tex.). The assay sensitivity was 0.8 μg/ml and the intrarun coefficient of variation was <5% in the concentration range of 0 to 50 μg/ml. Concentrations of aztreonam (23), cefepime (2), and ceftazidime (17) were determined by high-pressure liquid chromatography. The assays were linear in the ranges 0.5 to 100, 0.2 to 50, and 2.0 to 150 μg/ml. The coefficients of variation for the assays were <10%. All standards and samples were prepared and diluted as necessary in Mueller-Hinton broth supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter).

Statistical analysis. Analysis of variance was used to assess changes in $\log_{10}$ CFU/ml at 8 and 24 h by using Tukey's test for multiple comparisons. A P value of <0.05 was considered significant.

RESULTS

Pharmacokinetics. The pharmacokinetic parameters measured are shown in Table 2. The mean elimination half-life

TABLE 2. Pharmacokinetics for an in vitro model

Antimicrobial agent	Concn (μg/ml) in the following compartment[a]:				
	Central at:			Peripheral at:	
	5 h	24 h	$t_{1/2}$ (h)	3 h	24 h
Amikacin	77.9 ± 4.9	<0.8	2.1 ± 0	34.0 ± 8.8	<0.8
Aztreonam	94.0 ± 19.2	<2.0	1.9 ± 0.1	29.0 ± 2.1	2.4 ± 0.2
Cefepime	81.1 ± 18.0	<0.2	1.7 ± 0.1	36.3 ± 2.9	<0.2
Ceftazidime	102.8 ± 36.0	2.6 ± 0	2.2 ± 0.1	47.7 ± 12.1	4.4 ± 0.1

[a] Results are the means of two to eight experiments. All times are postbolus. $t_{1/2}$, half-life.

for all drugs was 2.0 ± 0.25 h in the central compartment. Concentrations of the antibiotics fell below the MICs for both organisms by 24 h in the peripheral compartment, with the exception of aztreonam and ceftazidime against ATCC 27853.

Pharmacodynamics. (i) Monotherapy drug regimens. Once-daily amikacin exhibited killing activity over the first 8 h superior to those of single β-lactam drug regimens against both strains ($P < 0.01$) and required an average of 1.9 ± 0.4 h to achieve a 99.9% reduction in $\log_{10}$ CFU/ml (Fig. 1; Table 3). Ceftazidime and cefepime had similar killing activities against ATCC 27853, with an average time of 5.3 ± 0.4 h to achieve 99.9% reductions in bacterial counts. Against strain 16690 (moderately susceptible to ceftazidime), cefepime required 8 h to reach a 99.9% reduction in $\log_{10}$ CFU/ml, and ceftazidime never achieved this degree of killing. Aztreonam did not achieve a 99.9% reduction in $\log_{10}$ CFU/ml for either strain. Killing activities following the administration of all second β-lactam doses were markedly reduced compared with those following the initial doses for both strains. Regrowth by 24 h was greatest for the amikacin regimen against both strains, but it was apparent in all monotherapy regimens, although no changes in susceptibilities were observed.

TABLE 3. Time required to achieve 99.9% reduction in $\log_{10}$ CFU/ml[a]

Regimen	Time (h) to achieve reduction with:	
	ATCC 27853	Strain 16690
AK	1.6	2.1
AZ	NA	NA
CP	5.5	8.0
CF	5.0	NA
AK + AZ	1.4	1.4
AK + CP	1.4	1.6
AK + CF	1.4	1.9
AZ + CP	6.0	6.4
AZ + CF	6.0	7.3

[a] Results are the means of two experiments. NA, never achieved. For other abbreviations, see Table 1, footnote *a*.

(ii) Combination drug regimens. Regardless of initial susceptibilities, similar killing activities were observed over the initial 8 h for all once-daily amikacin combinations against both strains (Fig. 2). The average time to achieve a 99.9% reduction in $\log_{10}$ CFU/ml was 1.5 ± 0.2 h. The two double β-lactam regimens demonstrated similar killing activities, with an average time to achieve 99.9% reductions in $\log_{10}$ CFU/ml of 6.4 ± 0.6 h. However, double β-lactam combinations demonstrated killing activities over the initial 8 h inferior to those of the amikacin-containing combinations ($P < 0.01$). As with that of monotherapy regimens, killing activity with double β-lactam regimens following the administration of the second β-lactam doses was markedly decreased compared with killing activity following the initial

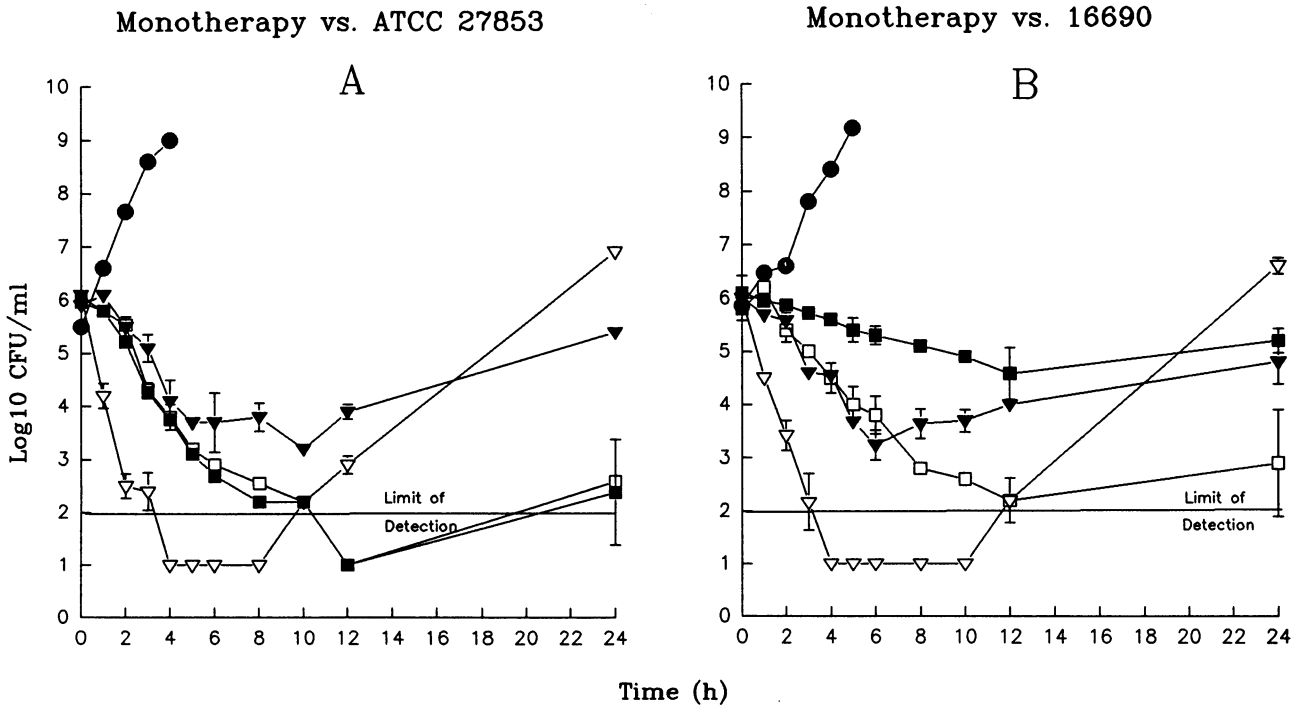

FIG. 1. Kill curves depicting $\log_{10}$ CFU/ml (mean ± standard deviation) versus time for monotherapy regimens against ATCC 27853 (A) and strain 16690 (B). ●, control; ▽, amikacin; ▼, aztreonam; □, cefepime; ■, ceftazidime. β-lactams were administered at 0 and 8 h.

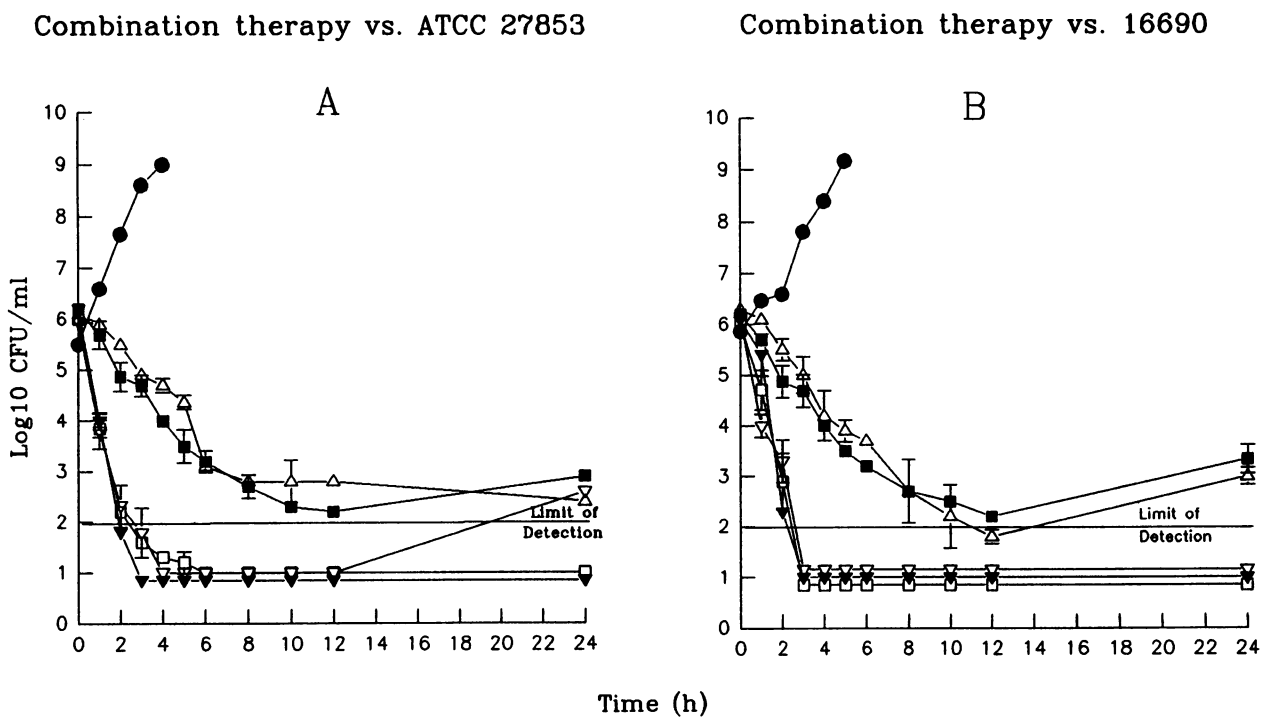

FIG. 2. Kill curves depicting $\log_{10}$ CFU/ml (mean ± standard deviation) versus time for combination regimens against ATCC 27853 (A) and strain 16690 (B). ●, control; ▽, amikacin-aztreonam; ▼, amikacin-cefepime; □, amikacin-ceftazidime; ■, aztreonam-cefepime; △, aztreonam-ceftazidime. β-lactams were administered at 0 and 8 h.

doses. Minimal regrowth was observed at 24 h for all double β-lactam combination regimens against both strains, with no changes in susceptibilities.

DISCUSSION

Infections caused by *P. aeruginosa* remain a therapeutic dilemma because of high rates of mortality, the development of resistance during appropriate therapy, and the lack of standardization for in vitro synergy testing (1). The majority of data regarding the optimal use of antipseudomonal antimicrobial agents has been derived from trials involving the treatment of febrile neutropenic patients (8, 24). Monotherapy is generally acceptable for the treatment of *P. aeruginosa* infections of the urinary tract, but controversy surrounds the appropriate therapy for infections involving other sites in both immunocompetent and neutropenic patients (9, 27).

In vitro susceptibility tests represent one of the most important influences on the selection of therapy for *P. aeruginosa* infections, although such tests should be only part of the decision-making process regarding therapy. Hilf et al., in a prospective, observational study, were unable to detect a significant correlation between MICs and MBCs and therapeutic outcome with 200 patients treated with various monotherapies and combination therapies for *P. aeruginosa* bacteremia (13). The authors did, however, conclude that the most important factor in determining outcome was early treatment with combinations of antipseudomonal agents. Equally important for determining antipseudomonal therapy should be consideration of the immunocompetence of and the site and source of infection in patients. In a unique, catheter-associated infection model, Vergeres and Blaser demonstrated decreased antibiotic bactericidal activity against adherent versus suspended bacteria, including *P. aeruginosa* ATCC 27853, although the bacteria were susceptible to the antibiotics by standard susceptibility testing (31).

This model investigation compared bactericidal activities against two strains of *P. aeruginosa* with different sensitivities. As expected, MIC and MBC data alone did not reliably predict killing activities. Once-daily amikacin regimens resulted in >4 log kill within the initial 8 h, with subsequent regrowth at 24 h beyond the starting inoculum, as reported previously (7, 16, 31). Haag et al. have reported regrowth with *P. aeruginosa* as a dilutional artifact in a one-compartment model with no antibiotic treatment and suggested that this was due to seeding of adherent bacteria (12). Although it is possible that a dilutional artifact may partly explain the regrowth, no change in susceptibility at 24 h was noted. Adherent bacteria typically represent a resident population with reduced sensitivities. Another possible explanation for the regrowth pattern demonstrated by amikacin may be the presence of slowly growing, aminoglycoside-resistant subpopulations harbored by many strains of *P. aeruginosa* as previously described by Gerber et al. (11). It is possible that our method of testing did not detect subpopulations, since the organisms were tested for susceptibility after overnight growth. Aztreonam did not achieve a 99.9% reduction in $\log_{10}$ CFU/ml for either strain. This result cannot be attributed to susceptibility data alone, although MICs of aztreonam were higher than those of the other β-lactams tested. As opposed to other β-lactam antibiotics which bind to multiple penicillin-binding proteins, aztreonam specifically binds only to PBP 3, which may account for its slower bactericidal activity. Cefepime was rapidly bactericidal against both strains, while ceftazidime never achieved a 99.9% reduction in $\log_{10}$ CFU/ml against strain 16690. All β-lactams demonstrated substantially less killing activity following the second

dose. This observation has been reported under similar experimental conditions, and the reason is unclear (31).

In vitro synergy tests consisting of checkerboard square and killing curves have been utilized to predict in vivo synergy. However, several limitations in the interpretation of these tests must be acknowledged. These tests are conducted under static conditions similar to MIC and MBC testing and do not account for the continually changing concentrations and penetrations of drug at the site of infection. Additionally, the effect of different dosing regimens cannot be determined. Zinner et al. have utilized a similar in vitro pharmacokinetic model to evaluate antibiotic combinations (33). Strong antagonism demonstrated by the checkerboard square technique for the combination of piperacillin and imipenem against *P. aeruginosa* was not apparent in the model. Piperacillin and amikacin demonstrated similar results for both tests. Azlocillin was reported to prevent the bacterial regrowth seen with netilmicin administered as monotherapy. In the present study, checkerboard square testing predicted synergy for all amikacin-containing combinations and additivity for the double β-lactam combinations. Every combination regimen which included once-daily amikacin, regardless of the organism's sensitivity to the β-lactam, required less than 2 h to achieve a 99.9% reduction in $\log_{10}$ CFU/ml (Fig. 2). All of the amikacin combinations, except for the combination of amikacin plus aztreonam against ATCC 27853, also resulted in no detectable regrowth over the 24-h period tested. However, this is believed to be an errant point. Initial susceptibility data (MICs and MBCs) for ceftazidime against strain 16690 did not appear important for killing activity or the prevention of regrowth when used in combination with once-daily amikacin in our model. Although the success of the aminoglycoside–β-lactam combinations is likely due to synergistic killing effects, other factors may also play a role. β-lactam suppression of aminoglycoside-resistant subpopulations, as mentioned earlier, may play an important role (11). Odenholt-Tornqvist et al. have demonstrated a subinhibitory antibacterial effect against both gram-negative and gram-positive bacteria following exposure to suprainhibitory doses of several β-lactam antibiotics (22).

The development of new β-lactam agents with greater activities against *P. aeruginosa* has renewed interest in double β-lactam combinations as a substitute for aminoglycoside combinations to possibly avoid the associated toxicity. Although synergism is rarely demonstrated for double β-lactam combinations in vitro, the results of clinical trials comparing double β-lactam combinations with aminoglycoside combinations report no difference in clinical responses (14). DeJongh et al. found the combination of moxalactam plus piperacillin as effective as moxalactam plus amikacin for febrile granulocytopenic patients with microbiologically documented infections (6). The combination of moxalactam and piperacillin, however, was noted to cause significantly less nephrotoxicity and ototoxicity. Of interest, amikacin in this study was administered every 6 h and may have contributed to the higher degree of nephrotoxicity. All double β-lactam combinations tested in our model required approximately 4 more hours than amikacin combinations to achieve 99.9% reductions in $\log_{10}$ CFU/ml (Fig. 2). While no regrowth was detected with amikacin combinations, except amikacin plus aztreonam, at 24 h, minimal regrowth was observed with all of the double β-lactam combinations.

Several limitations in the extrapolation of our in vitro model results to the in vivo setting exist. First, the model represents a general localized site of infection by barriers which freely allow passage of the antibiotic. Results may be different for infections such as meningitis, endocarditis, or deep-seated abscesses. Second, only two strains of *P. aeruginosa* with little variation in susceptibilities were studied. A wider variability in susceptibility against other study drugs may have further supported our conclusions regarding combination therapy with aztreonam or once-daily amikacin. Combination drug therapies were all administered simultaneously. This may bias the results, since this would not be the case in clinical practice. Lastly, only 2 doses of monobactam or β-lactam antibiotics were administered, whereas clinically 3 doses would have normally been given over the 24-h period. Although this limits interpretation of the monotherapy regimens, it lends strength to our conclusions about the abilities of the combination therapies to eliminate regrowth in the model at 24 h.

Overall, amikacin–β-lactam combinations demonstrated bactericidal activities superior to those of double β-lactam combinations over the initial 8 h. Data from our in vitro model support further investigation of once-daily amikacin in combination with β-lactams or aztreonam for the treatment of *P. aeruginosa*, including organisms with moderate susceptibilities.

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