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Optimization of Meropenem Minimum Concentration/MIC Ratio To Suppress In Vitro Resistance of *Pseudomonas aeruginosa*

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Suppression of resistance in a dense *Pseudomonas aeruginosa* population has previously been shown with optimized quinolone exposures. However, the relevance to β-lactams is unknown. We investigated the bactericidal activity of meropenem and its propensity to suppress *P. aeruginosa* resistance in an in vitro hollow-fiber infection model (HFIM). Two isogenic strains of *P. aeruginosa* (wild type and an AmpC stably derepressed mutant [MIC = 1 mg/liter]) were used. An HFIM inoculated with approximately 1×10^8 CFU/ml of bacteria was subjected to various meropenem exposures. Maintenance doses were given every 8 h to simulate the maximum concentration achieved after a 1-g dose in all regimens, but escalating unbound minimum concentrations (C_{min}s) were simulated with different clearances. Serial samples were obtained over 5 days to quantify the meropenem concentrations, the total bacterial population, and subpopulations with reduced susceptibilities to meropenem (>3× the MIC). For both strains, a significant bacterial burden reduction was seen with all regimens at 24 h. Regrowth was apparent after 3 days, with the C_{min}/MIC ratio being ≤1.7 (time above the MIC, 100%). Selective amplification of subpopulations with reduced susceptibilities to meropenem was suppressed with a C_{min}/MIC of ≥6.2 or by adding tobramycin to meropenem (C_{min}/MIC = 1.7). Investigations that were longer than 24 h and that used high inocula may be necessary to fully evaluate the relationship between drug exposures and the likelihood of resistance suppression. These results suggest that the C_{min}/MIC of meropenem can be optimized to suppress the emergence of non-plasmid-mediated *P. aeruginosa* resistance. Our in vitro data support the use of an extended duration of meropenem infusion for the treatment of severe nosocomial infections in combination with an aminoglycoside.

Bacterial resistance is a rapidly spreading and serious problem that threatens our therapeutic armamentarium. Given that the drug development process takes many years, it is imperative that the utilities of currently available agents be preserved through the judicious and optimal use of these agents. It has been shown that suboptimal dosing represents a selective pressure that is imposed on the bacteria and that facilitates the emergence of resistance (9, 11). On the other hand, all bacterial subpopulations are killed with optimal dosing, which results in the sustained suppression of both total and resistant populations over time. It has also previously been shown that the emergence of resistance in *Pseudomonas aeruginosa* could be suppressed by optimizing the exposure of quinolones (11, 28). However, it is less certain if the same is true for the β-lactam antibiotics.

The pharmacodynamics of β-lactams have been relatively well elucidated. The time above the MIC ($T > MIC$) of the pathogen has repeatedly been shown to be the pharmacodynamic variable most closely linked to bactericidal activity (2, 21). However, the breakpoint of optimal activity is controversial, and none of the studies to date have addressed the issue of resistance emergence. In addition, although the carbapenems are structurally related to the other β-lactams (penicillins and cephalosporins), their pharmacodynamic properties may be substantially different. Our preliminary data suggest that meropenem is less susceptible than other β-lactams to the inoculum effect (31). Spontaneous (non-plasmid-mediated) resistance selection in *Pseudomonas aeruginosa* may be suppressed with optimized meropenem exposure.

The prevalence of gram-negative bacterial resistance to β-lactams is on the rise, and the resistance is often mediated by stably derepressed AmpC β-lactamase production. This β-lactam resistance is broad spectrum and is not susceptible to clinically available β-lactamase inhibitors. These β-lactam-resistant isolates may exhibit a response to an antimicrobial exposure substantially different from that of their wild-type counterparts. The prolonged infusion of meropenem is being investigated clinically in a multicenter trial of nosocomial pneumonia (G. L. Drusano, personal communication). It is hoped that the pharmacodynamics of meropenem ($T > MIC$ and the minimum concentration [C_{min}]/MIC ratio) can be optimized by this innovative and practical dosing strategy. Meropenem is stable against enzymatic degradation by the AmpC β-lactamase, but it is unknown if the pharmacodynamic exposure for optimal bactericidal activity and resistance suppression remains unchanged. We explored the bactericidal activities of various meropenem exposures and their propensities to suppress *P. aeruginosa* resistance in an in vitro hollow-fiber infection model (HFIM). The objective of this study was to determine the in vitro pharmacodynamic exposure that would optimize the bactericidal activity of meropenem and its propensity to suppress spontaneous resistance emergence in *P. aeruginosa*.

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MATERIALS AND METHODS

Antimicrobial agent. Meropenem powder was supplied by AstraZeneca (Wilmington, DE). Tobramycin powder was purchased from Sigma-Aldrich (St. Louis, MO). A stock solution of each antimicrobial agent at 1,024 mg/liter in sterile water was prepared, aliquoted, and stored at −70°C. Prior to each susceptibility test, an aliquot of the drug was thawed and diluted to the desired concentrations with cation-adjusted Mueller-Hinton II broth (Ca-MHB; BBL, Sparks, MD).

Microorganisms. *P. aeruginosa* ATCC 27853 (American Type Culture Collection, Manassas, VA) and its isogenic daughter mutant (a stably derepressed AmpC β-lactamase producer) were used in the study. The isogenic daughter mutant was obtained by culturing a dense inoculum ($>1 \times 10^9$ CFU) of the standard parent strain on Mueller-Hinton agar (MHA) plates (Hardy Diagnostics, Santa Maria, CA) supplemented with ceftazidime at a concentration 8× the MIC. Chromosome-encoded β-lactamase (AmpC) overproduction was confirmed by a spectrophotometric assay using nitrocefin as the substrate with and without prior induction by imipenem, as described previously (14). The bacteria were stored at −70°C in Protect (Key Scientific Products, Round Rock, TX) storage vials. Fresh isolates were subcultured twice on 5% blood agar plates (Hardy Diagnostics) for 24 h at 35°C prior to each experiment.

Susceptibility studies. Meropenem and tobramycin MICs and minimum bactericidal concentrations (MBCs) were determined for both isolates in Ca-MHB by a broth macrodilution method described by the CLSI (formerly the National Committee for Clinical Laboratory Standards) (23). The final concentration of bacteria in each broth macrodilution tube was approximately 5×10^5 CFU/ml of Ca-MHB. Serial twofold dilutions of the drugs were used. The MIC was defined as the lowest concentration of drug that resulted in no visible growth after 24 h of incubation at 35°C in ambient air. Samples (50 μl) from clear tubes and the cloudy tube with the highest drug concentration were plated on MHA plates (Hardy Diagnostics). The MBC was defined as the lowest concentration of drug that resulted in ≥99.9% killing of the initial inoculum. Drug carryover effect was assessed by visual inspection of the distribution of colonies on medium plates. The studies were conducted in duplicate and were repeated at least once on a separate day.

Hollow-fiber infection model. The schematic of the HFIM system has been described previously (3). Drug was directly injected into the central reservoir to achieve the peak concentration desired. Fresh (drug-free) growth medium (Ca-MHB) was continuously infused from the diluent reservoir into the central reservoir to dilute the drug in order to simulate drug elimination in humans. An equal volume of drug-containing medium was removed from the central reservoir concurrently to maintain an isovolumetric system. Bacteria were inoculated into the extracapillary compartment of the hollow-fiber cartridge (Fibercell Systems, Inc., Frederick, MD); they are confined in the extracapillary compartment but are exposed to the fluctuating drug concentration in the central reservoir by means of an internal circulatory pump in the bioreactor loop. The optional absorption compartment of the system was not used.

Experimental setup. Overnight cultures of the isolates were diluted 30-fold with prewarmed Ca-MHB and were incubated further at 35°C until they reached late-log-phase growth. The bacterial suspension was diluted with Ca-MHB accordingly based on the absorbance at 630 nm; the bacteria (15 ml) were then inoculated into the hollow-fiber infection models at a concentration of approximately 1×10^8 CFU/ml. The high inoculum was used to simulate the bacterial load in a severe infection and to allow a resistant subpopulation(s) to be present at the baseline. The two bacterial isolates used were inoculated into two different hollow-fiber cartridges connected in series to ensure that they would be subjected to identical drug exposures. The experiment was conducted for 5 days in a humidified incubator set at 35°C. The systems were subjected to escalating meropenem exposures to simulate various steady-state pharmacokinetic profiles of unbound meropenem. Four meropenem regimens and a placebo were investigated, maintenance doses were given every 8 h to reattain the unbound maximum concentration (C_{max}) achieved after a 1-g dose (64 mg/liter) in all regimens. The regimens differed in escalating unbound C_{min} simulated with different clearances (Fig. 1A). The regimens investigated were guided by previous time-kill studies and pharmacokinetic-pharmacodynamic modeling (32). A sixth system was set up in which tobramycin was given in addition to meropenem. The simulated unbound tobramycin pharmacokinetic profile was used to mimic a clinically achievable exposure (peak concentration = 20 mg/liter, elimination half-life = 2 h) attained with administration once every 24 h.

Pharmacokinetic validation. Serial samples were obtained from the infection models at 0.1, 0.5, 1, 2, 4, 8 (on day 0), 48 (predosing), 48.5, 49, 50, 52, and 56 (on day 2) h. The meropenem concentrations in these samples were assayed by a validated bioassay method, as described below. For the combination regimen, serial samples were also obtained from the models at 0.5, 4, and 8 (on day 0 only) h. The tobramycin concentrations in these samples were assayed by a fluorescence polarization immunoassay (TDx; Abbott Laboratories, Abbott Park, IL). The concentration-time profiles were modeled by fitting a one-compartment linear model to the observations by using the ADAPT II program (7).

Bioassay. Meropenem concentrations were determined by a microbioassay with *Escherichia coli* ATCC 25922 as the reference organism. The bacteria were incorporated into 30 ml of molten cation-adjusted MHA (at 50°C) to achieve a final concentration of approximately 1×10^5 CFU/ml. The agar was allowed to solidify in 150-mm medium plates. A size 3 cork bore was used to create nine wells in the agar per plate. Standards and samples were tested in duplicate with 40 μl of the appropriate solution in each well. The meropenem concentrations in the standard solutions ranged from 0.125 to 128 mg/liter in Ca-MHB. The medium plates were incubated at 35°C for 24 h, and the zones of inhibition were measured. The assay was linear ($r^2 \geq 0.98$) by use of the zone diameter versus the log of the standard drug concentration. The intraday and interday coefficients of variation for all standards were <6% and <11%, respectively.

Microbiologic response. Serial samples were also obtained at the baseline and daily (predosing) in duplicate from each hollow-fiber system and submitted to quantitative culture to define the effects of various drug exposures on the total bacterial population and on the selection of resistant bacterial subpopulations. Before quantitative culture of the bacteria, the bacterial samples were centrifuged at $10{,}000 \times g$ for 15 min and reconstituted with sterile normal saline in order to minimize the drug carryover effect. Total bacterial populations were quantified by spiral plating 10× serial dilutions of the samples (50 μl) onto drug-free MHA plates. Subpopulations with reduced susceptibilities (resistance) were quantified by culturing them onto MHA plates supplemented with meropenem at a concentration of 3× the MIC of meropenem. Since susceptibility testing is performed in twofold dilutions and one tube (two times the concentration) difference is commonly accepted as a reasonable interday variation, quantitative cultures on drug-supplemented (at 3× the MIC) medium plates would allow the reliable detection of bacterial subpopulations with reduced susceptibilities. The medium plates were incubated at 35°C for up to 24 h (total population) and 72 h (subpopulations with reduced susceptibilities), and then the bacterial density from each sample was estimated with a CASBA-4 colony scanner and counter (Spiral Biotech, Bethesda, MD). The theoretical lower limit of detection was 400 CFU/ml.

Phenotypic screening of resistance mechanism. Subpopulations with reduced susceptibilities at the end of the experiment (three random isolates recovered from meropenem-supplemented plates) were stored. Determination of the susceptibilities of these isolates was repeated to document the presence of meropenem resistance. To provide insight into the likely mechanism of resistance, their susceptibilities (MICs) to a screening panel of antimicrobial agents (consisting of piperacillin, ceftazidime, imipenem, levofloxacin, and tobramycin) were determined by Etest (AB Biodisk, Piscataway, NJ), according to the manufacturer's instructions.

Demonstration of OprD loss. Based on the phenotypic resistance pattern compared to that of the parent isolate, porin protein OprD loss was suspected as the mechanism of resistance if meropenem resistance is coupled with resistance to imipenem but not to the other agents in the screening panel. The loss (reduced expression) of OprD in the resistant isolates was subsequently assessed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) of isolated outer membranes, as described previously (31). Briefly, lysis of the cells (overnight culture) was achieved by ultrasonication. Cell debris and residual cells were removed by centrifugation ($8{,}000 \times g$ at 4°C for 20 min). The protein contents of the membrane samples were determined with a bicinchoninic acid protein assay reagent kit (Pierce Biotechnology, Rockford, IL), following the manufacturer's instructions. The protein content of the samples was adjusted to 25 μg for the electrophoresis. Tris-glycine SDS buffer and 10% Tris-HCl gel (Bio-Rad Laboratories, Hercules, CA) were used. Strains PAO1 (OprD positive) and PAO1.2 (OprD negative) were used as positive and negative controls, respectively (26).

Demonstration of MexAB-OprM overexpression. Multidrug efflux pump overexpression was suspected as the mechanism of resistance if high-level meropenem resistance is coupled with resistance to levofloxacin (17); and the susceptibility to levofloxacin is enhanced in the presence of an efflux pump blocker, Phe-Arg β-naphthyl-amide dihydrochloride (MC-207,110; Sigma-Aldrich, St. Louis, MO) (15). Among the various efflux pumps that could have been overexpressed, MexAB-OprM overexpression was further suggested by the elevated

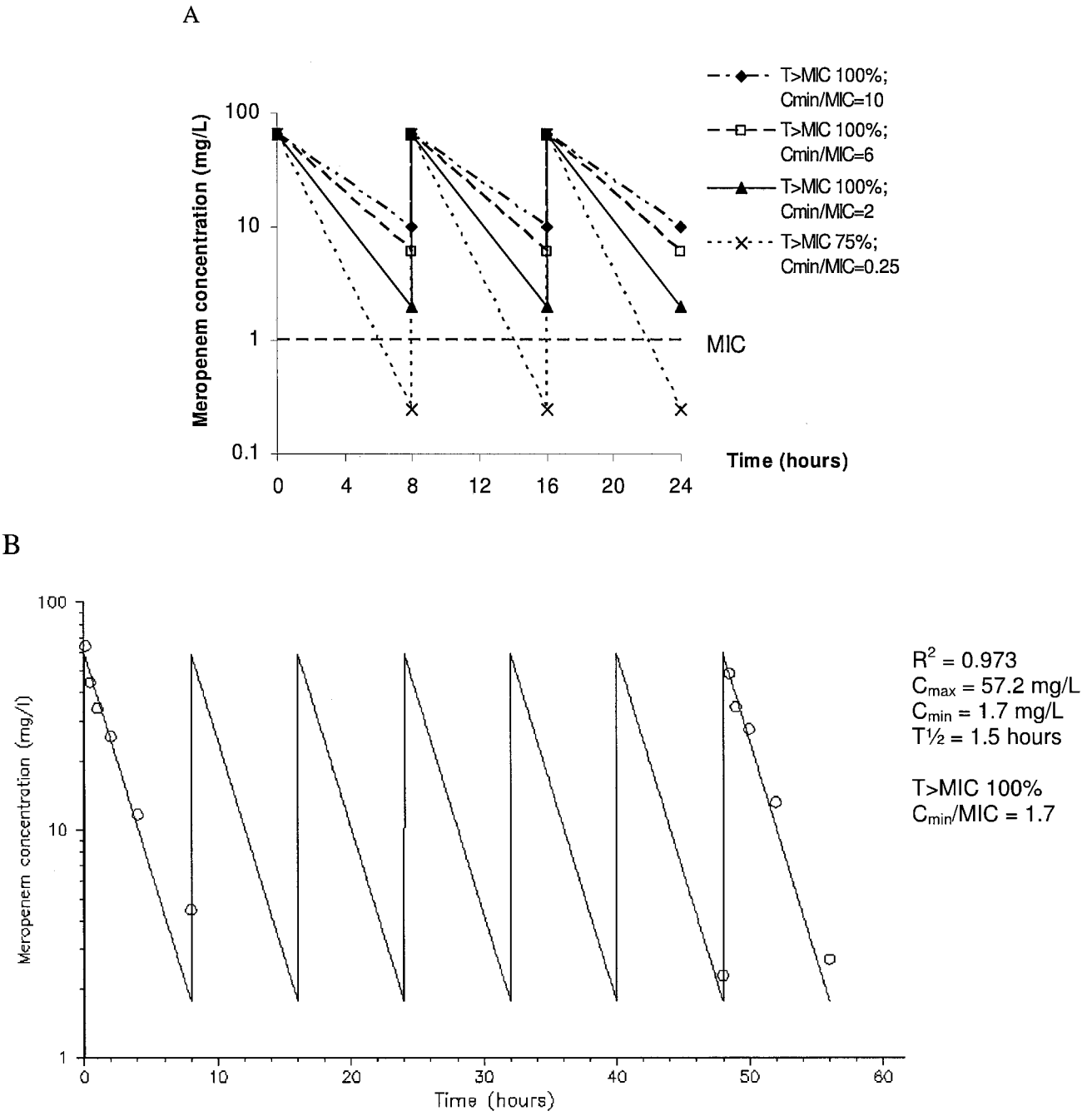

FIG. 1. Various pharmacokinetic simulations in the study. Target meropenem exposures (A); observed meropenem exposure (B); observed tobramycin exposure (C). T½, elimination half-life; $T >$ MIC is given as the percentage of the dosing interval.

MIC of carbenicillin (a specific MexAB-OprM substrate; determined only for selected isolates) but not of tobramycin (19). MexAB-OprM overexpression was confirmed by Western immunoblotting with MexB-specific antibodies, as described previously (20). *P. aeruginosa* strain OCR1 (a MexAB-OprM overproducer) was used as a positive control for MexAB-OprM overexpression (18). The repressor gene, *mexR*, for the *mexAB-oprM* operon of these resistant isolates was also amplified by PCR and sequenced, as reported previously (33). To assess the impact of the *mexR* mutation(s) on MexAB-OprM overexpression, the plasmid-borne wild-type *mexR* gene (GenBank accession number U23763) was introduced into the meropenem-resistant isolate(s) found to have a point mutations(s) in *mexR* by using a previously described triparental mating procedure (27), and susceptibility to carbenicillin was reassessed.

Studies with strains with *mexB* deletions. To provide more concrete evidence that MexAB-OprM overexpression was responsible for meropenem resistance, the *mexB* gene of the resistant isolates was knocked out by in-frame deletion, as described previously (10). Briefly, plasmid pRSP81 (a pEX18Tc derivative harboring the *mexB* deletion) was mobilized into the resistant isolates (27), and the transconjugants were selected on Luria agar (BBL) supplemented with tetracycline (at 3× the MIC) and imipenem (0.5 mg/liter; to counterselect *E. coli*). Tetracycline-resistant colonies were streaked onto sucrose (10%; wt/vol) containing Luria agar, and the *mexB* deletion in sucrose-resistant colonies was confirmed first by PCR and subsequently by reversal of susceptibility to meropenem, levofloxacin, and carbenicillin.

C

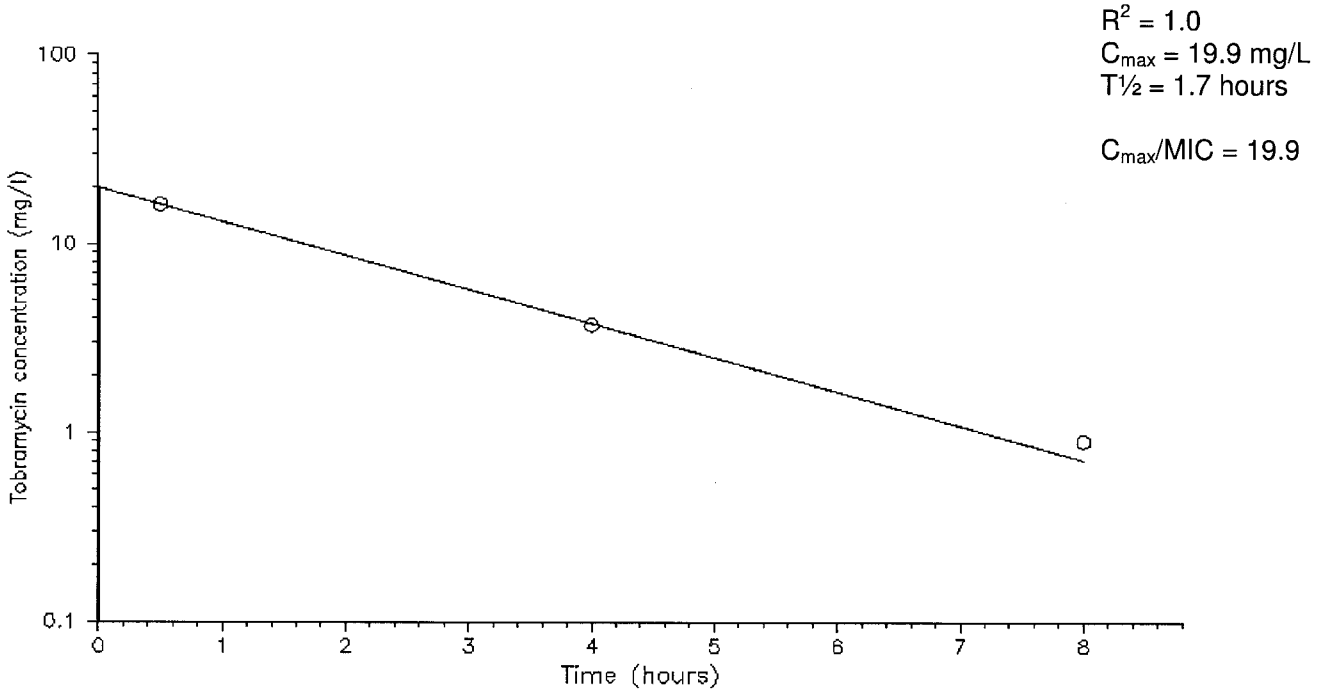

FIG. 1—*Continued*.

RESULTS

Microorganisms and susceptibility studies. The isogenic ceftazidime-resistant isolate (CAZ R2) was found to have stable derepression of β-lactamase (AmpC) production. The enzymatic activities of the wild-type isolate were found to be 24 (noninduced) and 4,225 (induced) U/mg of protein, respectively. In comparison, the enzymatic activities of the ceftazidime-resistant isolate were found to be 2,192 (noninduced) and 4,461 (induced) U/mg of protein, respectively. At the baseline the ceftazidime-resistant isolate expressed AmpC at a level approximately 100-fold more than that of the wild-type isolate. The meropenem and tobramycin MICs and MBCs for both isolates were 1 and 1 mg/liter.

Pharmacokinetic validation. All simulated meropenem and tobramycin exposures were satisfactory, and typical pharmacokinetic profiles are shown in Fig. 1B and C.

Microbiologic response. Ideally, the correlation of observations should be made to unbound (pharmacologically active) drug exposures (22). However, in view of the negligible protein binding of meropenem (8), we do not think that there will be a significant difference in our interpretation. The observed responses for both isolates (the wild type and the AmpC mutant) were similar, as shown in Fig. 2. Placebo did not exert any selective pressure on the bacteria; thus, the proportion of the resistant subpopulation to the total population remained low and constant over time (Fig. 2A). Suboptimal meropenem exposures reduced the bacterial burden significantly (>90%) within 24 h. However, they created a selective pressure by killing the susceptible populations preferentially; the emergence of resistance was observed over time due to selective amplification of a resistant subpopulation(s) (Fig. 2B and C). On the other hand, optimized (elevated) meropenem exposures resulted in the sustained suppression of all bacterial populations over time, as shown in Fig. 2D and E. Suppression of selective amplification of a resistant subpopulation(s) could also be achieved by using a combination of meropenem and tobramycin, as shown in Fig. 2F. This combination has previously been shown to be synergistic when the two drugs are used concurrently (30).

Mechanism of resistance. Imipenem cross-resistance and OprD loss were found to be the mechanism of resistance in all meropenem-resistant isolates, as shown in Fig. 3. In addition, MexAB-OprM overexpression was found to be the mechanism of meropenem resistance in three isolates (MR2, MR3, and MR5), as shown in Fig. 4. In these isolates, the susceptibilities to levofloxacin were dramatically enhanced (approximately 16- to 96-fold) in the presence of MC-207,110 (20 mg/liter). Furthermore, the susceptibilities to meropenem (4- to 8-fold), levofloxacin (4- to 8-fold), and carbenicillin (16- to 64-fold) were enhanced consequent to *mexB* deletion, confirming the contribution of MexAB-OprM overexpression to meropenem resistance. A point mutation in *mexR* (K71E) was found in only one mutant that overexpressed MexAB-OprM (mutant MR5), but complementation of the wild-type sequence did not result in a reversal of carbenicillin susceptibility.

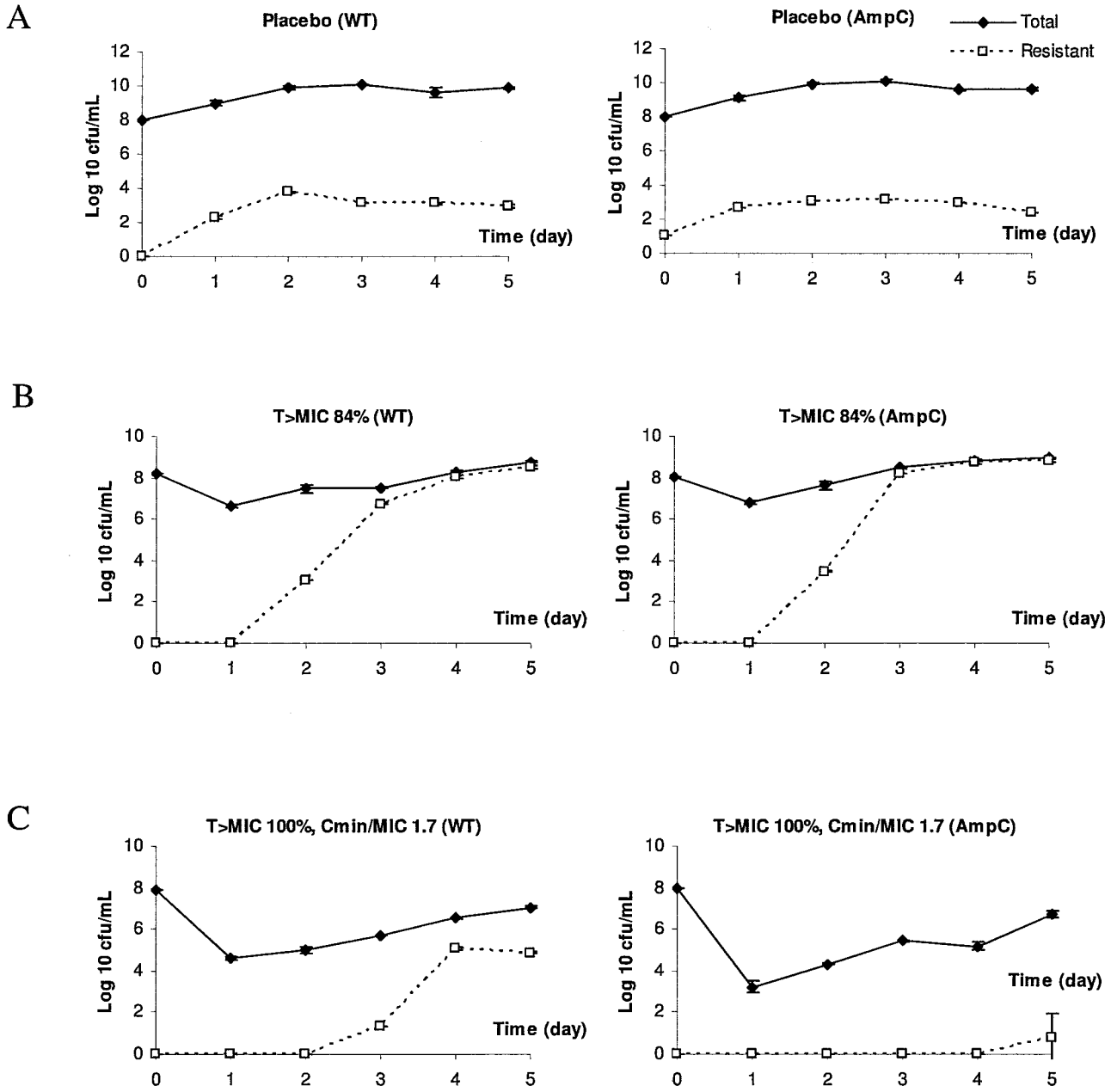

FIG. 2. Observed microbiologic responses to various meropenem exposures. Data are presented as the means ± standard deviations of the bacterial burden. WT, wild type; AmpC, ceftazidime-resistant (AmpC) mutant.

DISCUSSION

The prevalence of gram-negative bacterial resistance is on the rise. More importantly, an alarmingly high prevalence rate of multidrug resistance in gram-negative bacteria had been reported (13), and it had been escalating steadily (24). Very few agents designed to target multidrug resistant gram-negative bacteria are in the advanced stage of development, and none is expected to be available for clinical use in this decade. As widely appreciated as the magnitude of this problem may be, the traditional approach to the development of a new antimicrobial agent(s) is unlikely to meet this critical need. The traditional approach of drug development has emphasized the discovery of new agents, but relatively little attention is paid to the impact of dosing regimens on the emergence of resistance.

In this study we explored the feasibility of using various meropenem exposures to suppress spontaneous resistance in two strains of *P. aeruginosa*. In all active treatment regimens, identical peak concentrations were used since the bactericidal activity of meropenem was believed to be partially concentration dependent; high peak concentrations did not contribute significantly to enhanced killing (32). In contrast, the effect of escalating C_{min}s (resulting from prolonged or continuous infusion) was investigated. The specific meropenem exposures and duration of treatment investigated in the study were guided by

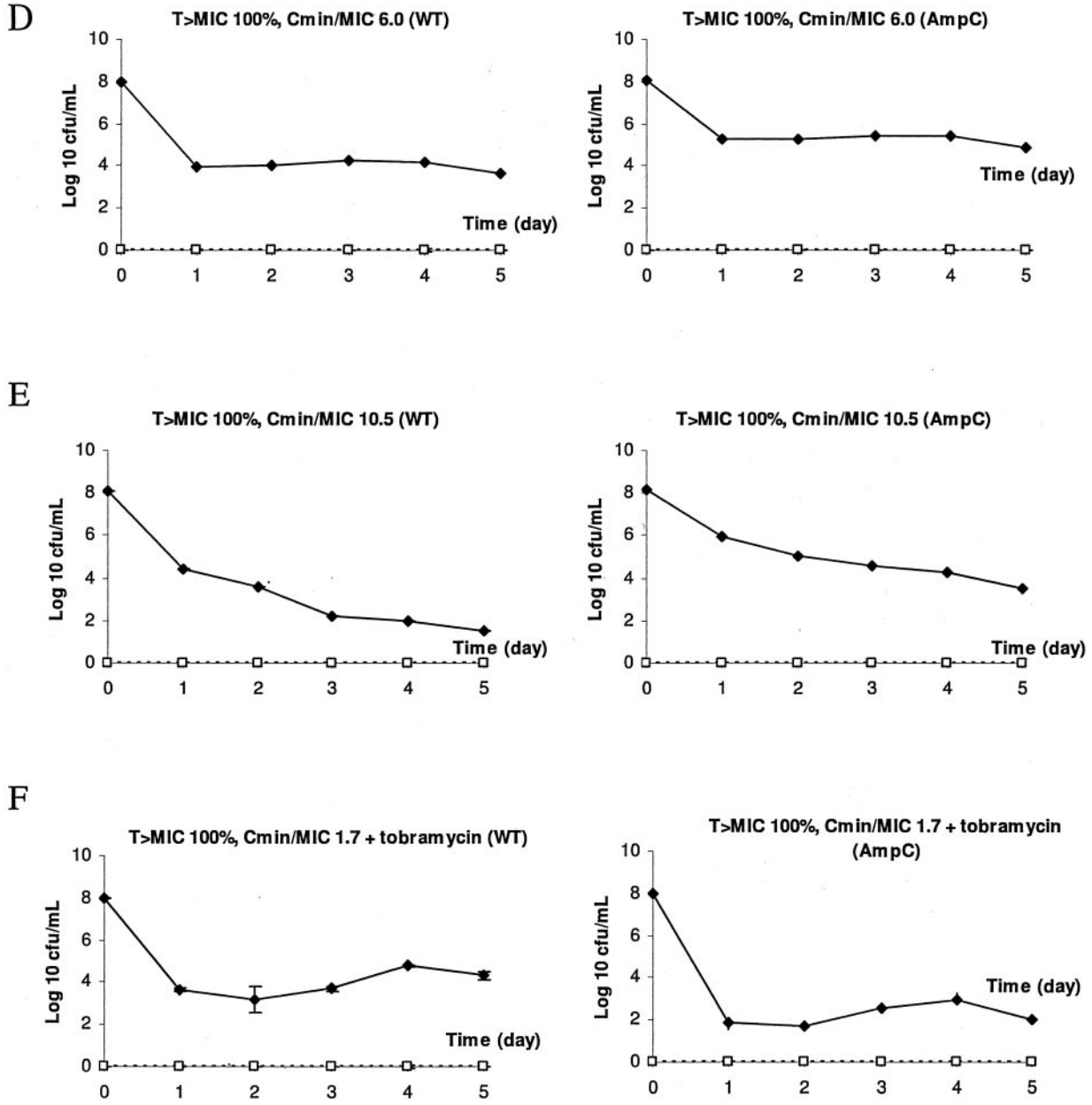

FIG. 2—*Continued*.

computer modeling and simulations (data not shown). A high inoculum (15 ml of approximately 1×10^8 CFU/ml) was used to simulate the bacterial burden in severe infections, such as nosocomial pneumonia and empyema. In view of the fact that the inoculum was greater than the natural mutation frequency of resistance (commonly reported to be 1 in 10^7 to 10^8), there was a higher probability of a resistant subpopulation being present at baseline. The experimental conditions represent a very conservative situation in the clinical setting (neutropenia and high bacterial burden).

Many pharmacodynamic studies were of limited duration (≤24 h) and were not designed to investigate the effects of drug exposures on resistance suppression (2, 6, 21, 25). There might be inadequate time for selective amplification of the resistant subpopulation to be apparent, even given a suboptimal exposure. We have shown previously that meropenem is less susceptible than piperacillin and ceftazidime to the inoculum effect; thus, it may be possible to optimize meropenem exposure to suppress resistance in a dense population of *P. aeruginosa* (31). However, a longer duration of observation with repeated dosing might be necessary to fully evaluate the propensity of a dosing regimen (dose and dosing frequency) to suppress resistance. As a result, the experiments were conducted over 5 days. Furthermore, this study design also simulates a treatment course of infection more closely.

It was evident that all treatment regimens resulted in a

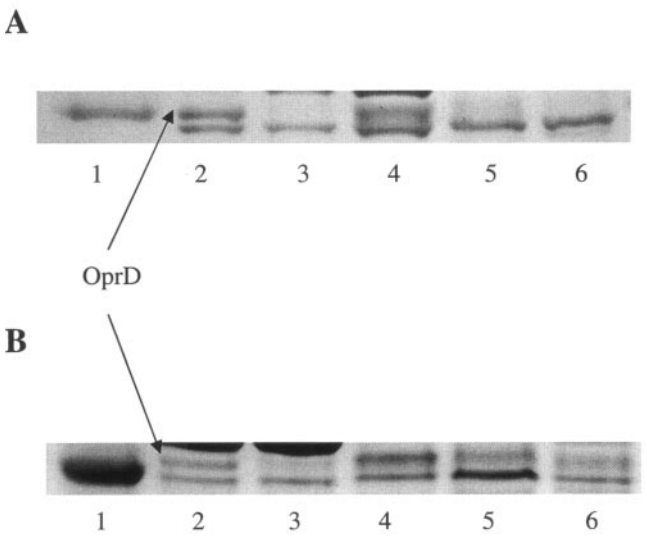

FIG. 3. SDS-PAGE illustrating reduced expression of OprD in isolates recovered from meropenem-supplemented plates. (A) Lane 1, molecular marker (47 kDa); lane 2, PAO1 (OprD positive); lane 3, PAO1.2 (OprD negative); lane 4, *P. aeruginosa* ATCC 27853 (wild type); lane 5, MR1; lane 6, MR2. (B) Lane 1, molecular marker (47 kDa); lane 2, PAO1 (OprD positive); lane 3, PAO1.2 (OprD negative); lane 4, CAZ R2; lane 5, MR4; lane 6, MR5. MR1 (placebo regimen) and MR2 (C_{min}/MIC = 0.5) were derived from *P. aeruginosa* 27853; MR4 (placebo regimen) and MR5 (C_{min}/MIC = 0.5) were derived from CAZ R2.

significant reduction of the bacterial burden in the first 24 h (Fig. 2B to F). However, they differed in their propensities to suppress resistance beyond 24 h and with repeated dosing. The emergence of resistance during drug exposure could be due the amplification of a resistant mutant(s) present at the baseline. The drug exposure necessary to suppress resistance emergence (C_{min}/MIC = 6) appeared to be consistent with those from previous studies, which suggested that the bactericidal activities of β-lactams were maximized at 4× to 6× the MIC (4, 16, 29), but greater than the widely accepted optimal pharmacodynamic threshold(s) for the β-lactams ($T >$ MIC = 40 to 50%). It is important to recognize that our study design is different from those of most pharmacodynamic studies. The primary end point of this study was resistance suppression over 5 days rather than killing over 24 h. As a result, the threshold drug exposure necessary to achieve the end point may be different. Similar observations have been made with the fluoroquinolones (1).

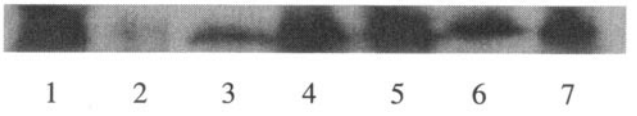

FIG. 4. Western immunoblotting illustrating the overexpression of efflux pumps in isolates recovered from meropenem-supplemented plates. Lane 1, OCR1 (MexAB-OprM overproducer); lane 2, PAO1 (wild type); lane 3, *P. aeruginosa* ATCC 27853 (wild type); lane 4, MR2; lane 5, MR3; lane 6, CAZ R2; lane 7, MR5. MR2 (C_{min}/MIC = 0.5) and MR3 (C_{min}/MIC = 1.7) were derived from *P. aeruginosa* 27853; MR5 (C_{min}/MIC = 0.5) was derived from CAZ R2.

We found C_{min}/MIC to be a useful pharmacodynamic parameter. As illustrated in Fig. 1A, $T >$ MIC (as a percentage of the dosing interval) may be limited by a ceiling effect of 100%; various drug exposures with C_{min}/MIC ratios ≥1 would have identical $T >$ MIC values (100%). Thus, analysis by the use of $T >$ MIC may not be satisfactory if the breakpoint for a favorable outcome is beyond 100%. On the other hand, C_{min}/MIC appears to be a more flexible parameter which is not constrained by such a limitation and allows greater drug exposures to be quantitatively expressed.

We recognize that the meropenem pharmacodynamic exposure necessary to suppress resistance may not be readily achievable in humans by the use of conventional dosing. We previously found that meropenem and tobramycin exhibited synergistic killing when they were used in combination (30). Therefore, we examined the feasibility of adding an aminoglycoside to lower the meropenem pharmacodynamic threshold necessary to suppress resistance. As shown in Fig. 2F, regrowth and selective amplification of the resistant subpopulation were not apparent over 5 days when a clinically relevant regimen of tobramycin was added to meropenem (C_{min}/MIC = 1.7). This was in direct contrast to the findings presented in Fig. 2C, where regrowth was evident beyond 24 h by using an identical meropenem exposure. The meropenem C_{min} of 1.7 mg/liter can be clinically achieved by a prolonged (3-h) infusion (5). By using this dosing strategy, suppression of spontaneous pseudomonal resistance is likely to be achieved in immunocompetent patients when it is used in combination with an aminoglycoside.

We also noticed that stable derepressed AmpC production did not appear to affect the activity of meropenem, as reflected in the MIC and the level of pharmacodynamic exposure required to suppress resistance emergence. As we had anticipated, the deletion of an outer membrane porin (OprD) was the most common mechanism of resistance. Cross-resistance to imipenem only (but not to other agents) was observed. However, in contrast to our previous observations (31), efflux pump overexpression (e.g., MexAB-OprM) appeared to play an important role in some OprD-deficient isolates as well. Our observations were consistent with those reported previously (12). The specific risk factor(s) that favors the emergence of different mechanisms of resistance under suboptimal meropenem exposures is under investigation. In summary, the C_{min}/MIC of meropenem could be optimized to suppress the emergence of non-plasmid-mediated resistance in *P. aeruginosa*. Our data support the use of an extended duration of meropenem infusion for the treatment of severe nosocomial infections when it is used in combination with an aminoglycoside.

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