The Combination of Meropenem and Levofloxacin Is Synergistic with Respect to both Pseudomonas aeruginosa Kill Rate and Resistance Suppression

Arnold Louie, Caroline Grasso, Nadzeya Bahniuk, Brian Van Scoy, David L. Brown, Robert Kulawy, and G. L. Drusano*

Ordway Research Institute, Albany, New York

Received 16 January 2010/Returned for modification 16 March 2010/Accepted 28 March 2010

New approaches are needed for the treatment of Pseudomonas aeruginosa infections. All available single agents are suboptimal, especially for resistance suppression. Classical β-lactam/aminoglycoside combinations are not used often enough at least in part because of concern for nephrotoxicity. We evaluated the combination of meropenem and levofloxacin against the P. aeruginosa PAO1 wild type and its isogenic MexAB pump-overexpressed mutant. The drugs were studied using an in vitro hollow-fiber pharmacodynamic infection model. There were 16 different regimens evaluated for both isolates. Both total population and resistant subpopulations were quantified. Drug concentrations were measured by liquid chromatography-tandem mass spectrometry (LC–MS-MS). The impact of monotherapy versus that of combination therapy for attainment of a 3-log cell kill and for resistance suppression was examined using Kaplan-Meier analysis. Drug exposures were calculated by fitting the concentration-time data using the ADAPT II package of programs. For both isolates, monotherapy allowed resistance emergence with all but the largest exposure or with all exposures. In contrast, there was no resistance emergence with any combination regimen. Kaplan-Meier analysis showed significant differences in time to attainment of a 3-log cell kill as well as time to resistance emergence for monotherapy and combination therapy for both isolates, in favor of the combination regimens. Determination of the pharmacodynamic indices associated with resistance suppression demonstrated a 2- to 3-fold reduction with the use of combinations. Combination therapy with meropenem and levofloxacin provides a significantly faster time to attain a 3-log cell kill and significantly better resistance suppression than does either monotherapy. This combination should be evaluated in a clinical trial.

Pseudomonas aeruginosa causes severe infections resulting in considerable mortality and morbidity, particularly in intensive care unit patients with ventilator-associated pneumonia. No single agent is adequate to provide a cell kill sufficient to allow an optimal clinical outcome and simultaneously suppress amplification of less susceptible subpopulations of organisms, when examined by Monte Carlo simulation across a broad range of exposures. For instance, this laboratory has demonstrated that levofloxacin can suppress less-susceptible-subpopulation amplification when an exposure of an AUC/MIC ratio (area under the concentration-time curve divided by the MIC) of 157 was attained (10). When Monte Carlo simulation was employed to examine how often such a ratio was attained in patients treated with 750 mg levofloxacin given once daily and across a distribution of levofloxacin MIC values, only 61% were expected to achieve such an exposure for a collection of Pseudomonas aeruginosa isolates. When this method was employed with ciprofloxacin for Pseudomonas in patients with hospital-acquired pneumonia, the outcomes correlated quite closely with resistance emergence rates observed in clinical trials (8, 10, 12).

Consequently, it is important to explore combination therapy to attain the twin goals of rapid reduction in organism burden (rapid kill rate, making clinical success likely) and suppression of amplification of less susceptible organism subpopulations. The classical combination of a β-lactam and an aminoglycoside has been used for these purposes (13). Our laboratory has previously examined the combination of meropenem and tobramycin against both the PAO1 strain of Pseudomonas aeruginosa and its isogenic MexAB pump-overexpressed strain in a checkerboard kill curve evaluation (7).

While resistance suppression was demonstrated, the cell kill interaction was additive and not synergistic. Further, it was more difficult to suppress tobramycin resistance than we had expected, particularly with the MexAB efflux pump-overexpressed strain, even though tobramycin is not known to be removed from P. aeruginosa by MexAB pumps but rather by MexXY pumps. We therefore decided to examine a different drug combination.

West and colleagues (18) demonstrated in a randomized clinical trial of levofloxacin for nosocomial pneumonia that in 17 patients infected with Pseudomonas aeruginosa and treated with the combination of a β-lactam and levofloxacin (750 mg daily) there was no case of emergence of resistance. To put this into perspective, when ciprofloxacin was administered as a single agent to a patient population with nosocomial pneumonia at a dose and schedule of 400 mg intravenously (i.v.) every 8 h (q8h), there was a 33% rate (12/36) of emergence of resistance to therapy (8).

Given this unexpected clinical finding, we wished to explore the combination of meropenem (as the β-lactam) and levo-
floxac in against the PAO1 wild-type (WT) organism and its MexAB pump-overexpressed isogenic mutant in our hollow-fiber infection model (HFIM) for a clinically relevant 14-day administration period.

MATERIALS AND METHODS

Microorganisms. The Pseudomonas aeruginosa strain PAO1 and its MexAB pump-overexpressed isogenic mutant were the kind gift of Keith Poole, Queens University, Ontario, Canada. MIC values of both meropenem and tobramycin were determined by CLSI macrobroth methodology (4). The subpopulation densities to resistance were estimated by plating 5 ml each (overnight growth) of the wild-type PAO1 strain and its MexAB pump-overexpressed isogenic mutant onto agar containing 5× the baseline MIC or 3× the baseline MIC of meropenem or levofloxacin, respectively. The concentration of microbes in the bacterial suspension was determined by quantitative cultures, and the ratio provided the estimate of the subpopulation density to resistance (15, 16). This calculation was done on at least three occasions. The concentrations in the agar screening for resistance were chosen because the most common mutation resulting in an increased MIC of meropenem is the stable downregulation of oprD, which results in a change in MIC of 4 to 8-fold, and that for levofloxacin in either the topoisomerase genes or an efflux pump overexpression that usually causes a 3- to 4-fold change in the MIC (data not shown). At least three colonies were randomly picked from each resistance plate and tested for a change in the MIC from baseline.

Hollow-fiber infection model. The hollow-fiber bioreactor system (HFS) was first described for use as a pharmacodynamic (PD) system for bacteria by Blaser et al. (3) and for HIV pharmacodynamic studies by Bicello et al. (1). A schematic diagram of the system and a description of its use were presented previously in our study of Mycobacterium tuberculosis (9). Here, meropenem and/or levofloxacin was injected directly into the central reservoir every 8 h (meropenem) or daily (levofloxacin). Levofloxacin was administered over a period of 1 h to achieve the desired concentration-time profiles; for meropenem, the infusion time was 1 h for the WT strain of 4 to 6 h for the pump-overexpressed strain. This was done because preliminary experiments demonstrated that the upregulation of MexAB required considerably more meropenem exposure and that this could be accomplished by a prolonged infusion (greater time > MIC). In experiments where the two drugs were administered together, the quite disparate half-lives of meropenem (circa 1 h) and levofloxacin (circa 8 h) were achieved simultaneously using the approach of Blaser (2).

Dose-response studies. The inoculum was prepared by growing 3 medium-sized colonies of P. aeruginosa in Ca–Mueller-Hinton broth (MH) overnight at 35°C. Hollow-fiber systems were maintained at 35°C in a humidified incubator. Approximately 15 ml of bacterial culture in late-log-phase growth (1.5 × 10^9 CFU/ml) was infused into each of 16 cartridges, one for each nominal dosing regimen. For levofloxacin monotherapy, nominal doses of 750 mg, 1,000 mg, and 1,250 mg once daily were simulated for the WT isolate. For the MexAB isogenic mutants, 1,500 mg daily was substituted for 1,250 mg daily. For meropenem monotherapy, doses of 1 mg, 2 mg, and 3 mg given every 8 h as a 1-h infusion were simulated for the WT isolate. For the MexAB isolate, the infusion of meropenem was prolonged to 4 h. The doses used for combination regimens are as follows. The 750-mg levofloxacin dose was combined with 1 mg, 2 mg, and 3 mg of meropenem every 8 h for both isolates. The 1,000-mg levofloxacin dose was combined with meropenem at doses of 1 mg, 1.5 mg, and 2 mg every 8 h for both isolates. The levofloxacin doses of 1,250 mg for the WT isolate and 1,500 mg for the MexAB isolate were each combined with 1 mg, 1.5 mg, and 2 mg of meropenem every 8 h. These exposures were used to simulate steady-state human pharmacokinetics of unbound drugs. Experimentally attained meropenem and levofloxacin concentration exposures were determined by quantifying drug concentrations using validated liquid chromatography-tandem mass spectrometry (LC–MS–MS) methods, with samples taken from the central bioreactor loop at 14 to 18 time points over the first 48 h for monotherapy and at 16 to 18 time points each for both agents in combination regimens over the first 48 h. At 0 (baseline), 0.2, 1, 2, 3, 4, 6, 8, 10, 13, and 14 days of the experiment, samples of the bacterial cultures were obtained from the cartridges, washed, and resuspended in normal saline in order to minimize drug carryover effect. Serially diluted samples were quantitatively cultured onto drug-free Mueller-Hinton II agar plates to enumerate the total bacterial population to our limit of detection, circa 30 CFU/ml. A portion of the bacterial suspension was also quantitatively cultured onto agar that was supplemented with either meropenem at 5× the baseline MIC or levofloxacin at 3× the baseline MIC for each isolate in order to assess the effect of each regimen on the less susceptible bacterial populations.

Plates containing media were incubated at 35°C for 24 h (total population) or 72 h (resistant subpopulations) before results were read. The MICs of meropenem and levofloxacin were determined for a subset of the colonies that grew on drug-supplemented agar to confirm the emergence of resistance.

Pharmacokinetic methods. Concentration-time profiles were analyzed employing maximum-likelihood estimation. The identification module of the ADAPT II package of programs of D’Argenio and Schumitzky (5) was used. As for controlled infusion pumps (dove the profile, a 3-compartment open model with zero-order input and first-order elimination was employed.

The period of time during which the free drug concentration exceeded the MIC (free drug time > MIC) was estimated by integrating the following differential equation, which was a system output:

\[
\text{IF} \{[X(1)\text{Vol.GE.MIC}] \text{ THEN} \ dx(3)/dt = 1.0 \ \\
\text{ELSE} \ dx(3)/dt = 0.0 \ \\
\text{ENDIF}
\]

where X(1) is the amount of drug in the central compartment, Vol is the volume of the central compartment, MIC is the MIC of the appropriate drug for the pathogen being studied, and GE is “greater than or equal to.” The AUC from time zero to time t (AUC_{0–t}) was calculated by integration.

The differential equation was written as dx(t)/dt = X(1)/Vol, with the same definitions given above, and the system output was Y(t) = X(2), which integrates the \text{AUC}_{0–t}.

LC–MS–MS methods for meropenem and levofloxacin. Mueller-Hinton II broth pharmacokinetic simulation samples were diluted with high-pressure liquid chromatography (HPLC) water (0.050 ml sample into 1.00 ml water) and analyzed by high-pressure liquid chromatography–tandem mass spectrometry (LC–MS–MS) to determine meropenem and levofloxacin concentrations. The LC–MS–MS system was comprised of a Shimadzu Prominence HPLC system and an Applied Biosystems/MDS Sciex API5000 LC–MS–MS instrument.

Chromatographic separation was performed using a Thermo Scientific Hypersil Gold C18 column (5 μm, 150 by 4.6 mm) and a mobile phase consisting of 80:20:0.1% formic acid in water:0.1% formic acid in acetonitrile at a flow rate of 0.75 ml/min.

Meropenem and levofloxacin concentrations were determined concurrently using LC–MS–MS, monitoring the MS–MS transitions m/z 384 → m/z 141 for meropenem and m/z 362 → m/z 261 for levofloxacin. The analysis run time was 4.5 min. The assay was linear over a range of 0.50 to 200 μg/ml (r^2 > 0.995) for meropenem and a range of 0.050 to 2.00 μg/ml (r^2 > 0.993) for levofloxacin. The interday coefficients of variation (CVs) for the quality control samples (containing both meropenem and levofloxacin), analyzed in replicates of three at each concentration on each analysis day, ranged from 3.07 to 7.22% for meropenem and 3.42 to 8.88% for levofloxacin. Accuracies (%REC) for these same quality control samples ranged from 97.1 to 108% for meropenem and from 94.0 to 105% for levofloxacin.

Statistical methods. Time to resistance emergence and time to achieve a 3-log-CFU/ml reduction in bacterial burden were both examined in both sets of experiments by using a Kaplan-Meier product limit estimator. Results were stratified by single-agent versus combination agent therapy. Because there is some confounding between the time to attainment of a 3-log-CFU/ml reduction in bacterial burden and the time to resistance emergence, monotherapy regimens with resistance emergence had the time to a 3-log-CFU/ml cell kill right censored at the time that resistance amplification occurred if there was no achievement of the kill endpoint over the period of observation.

RESULTS

MIC values. The MICs for the PAO1 wild-type strain were 0.5 mg/liter for meropenem and 1.0 mg/liter for levofloxacin. For the efflux pump-overexpressed mutant, the MIC value of both agents was 2.0 mg/liter.

Mutation frequency to resistance. For meropenem, the subpopulation density to resistance at 5× the baseline MIC ranged from −7.22 to −8.97 log CFU for both isolates. For levofloxacin, the subpopulation density to resistance at 3× the baseline MIC was −5.88 to −6.24 log CFU for the WT isolate.
or \( < -8.69 \) log CFU for the MexAB pump-overexpressed isolate.

**Documentation of desired concentration-time curves.** The concentration-time curves in all instances were quite close to nominal. (The nominal AUC and half-life for a 1-mg meropenem dose were 96.9 mg \( \cdot \) hr/liter and 1 h, respectively. For a 750-mg levofloxacin dose, these values were 78.9 mg \( \cdot \) hr/liter and 7.5 h. All other doses used alone and in combination were scaled to these values.) Data for all regimens are available upon request.

**Effects of monotherapy regimens on total-population and resistant-population burdens for the WT and MexAB pump-overexpressed *P. aeruginosa* isolates.** For both monotherapy regimens, rapid resistance emergence was seen for all regimens except at the highest dose of each drug for the WT isolate. All monotherapy arms, except for the highest dose of meropenem, also demonstrated outgrowth for the MexAB efflux pump-overexpressed isolate. The effects of the levofloxacin and meropenem monotherapies on the total-population burden for the WT isolate are shown in Fig. 1A and B, and those for the MexAB pump-overexpressed isolate are shown in Fig. 1C and D. Resistance emergence has an impact on the attainment of a 3-log-CFU/ml cell kill for the monotherapy regimens. Figure 2 displays the resistant counts for the monotherapy regimens for the WT isolate. The regrowth in the total counts for 4 of the 6 monotherapy regimens seen in Fig. 1 can therefore be explained by the emergence of resistance. Monotherapies using the highest dose of levofloxacin and the highest dose of meropenem were able to suppress the resistant-subpopulation amplification for the WT isolate.

Figure 3 displays the resistant counts for the monotherapy regimens for the MexAB isolate. In contradistinction to the results derived with the WT isolate, all but the monotherapy arms using meropenem at 3,000 mg q8h against the MexAB pump-overexpressing *P. aeruginosa* strain had breakthrough growth due to resistant mutants. It should also be noted that meropenem was administered as a 4-h prolonged infusion in this experiment. Previous experiments using meropenem alone demonstrated consistent resistance emergence at this dose administered as a standard 1-h infusion (data not shown), which prompted the prolonged infusion for this isolate in this experiment.

**Effects of combination therapy regimens on total-population and resistant-population burdens for the WT and MexAB isolates.** Figure 4 shows the effects of combination therapy on the WT isolate (Fig. 4A to C) and the MexAB isolate (Fig. 4D to F). Combination therapy has a major impact, with all regimens (even the lowest dose of each drug) resulting in sterilization of the hollow-fiber systems. All treatment arms were sterilized by day 10 or before. At day 14, all units reading as sterile at 10- and 13-day time points (for both monotherapy and combination therapy) had their total volumes removed, washed, and cultured to document sterility. All combination therapy arms and the high-dose-levofloxacin arm for the WT isolate were documented as sterile.
Examination of drug interaction with regard to cell kill and resistance suppression for the WT isolate. Figure 5A shows a Kaplan-Meier plot comparing the abilities of monotherapy and combination therapy to induce a 3-log-CFU/ml cell kill for the WT isolate. Combination therapy generates a 3-log-CFU/ml cell kill in all instances and does so in 1 day or less. This is significantly different from the results for monotherapy ($P = 0.0002$; Breslow-Gehan test). Figure 5B shows this contrast for the endpoint of resistance emergence. Here again, combination therapy is significantly better than monotherapy at resistance suppression, with no resistance emerging for the combination regimen ($P = 0.005$; Breslow-Gehan test). Results from log rank tests were also highly significant for both contrasts.

Combination therapy was significantly better than monotherapy for both endpoints for the WT isolate.

Examination of drug interaction with regard to cell kill and resistance suppression for the MexAB efflux pump-overexpressed isolate. Figure 6A shows a Kaplan-Meier plot comparing the abilities of monotherapy and combination therapy to induce a 3-log-CFU/ml cell kill for the MexAB isolate. Combination therapy generates a 3-log-CFU/ml cell kill in all instances and does so in 1 day or less. This is significantly different from the results for monotherapy ($P = 0.004$; Breslow-Gehan test). Figure 6B shows this contrast for the endpoint of resistance emergence. Here again, combination therapy is significantly better than monotherapy at resistance suppression, with
no resistance emerging for the combination therapy ($P = 0.004$; Breslow-Gehan test). Results from log rank tests were also highly significant for both contrasts.

Combination therapy was significantly better than monotherapy for both endpoints for the MexAB pump-overexpressed isolate.

Examination of pharmacodynamic indices for monotherapy and combination therapy regimens. We examined the concentration-time profiles for the monotherapy and combination therapy regimens and used suppression of resistance as the index of success. Table 1 shows the PD indices and values for the monotherapy regimens that failed and succeeded for both the WT isolate and the MexAB pump-overexpressed isolate.

For the WT isolate, meropenem alone for all regimens had $100\%$ free drug time $>\text{MIC}$. We therefore used the free drug $C_{\text{min}}/\text{MIC}$ ratio (minimum concentration of drug in serum divided by the MIC) as a PD index, without an upper bound. The 1-h-infusion regimen of 2,000 mg every 8 h failed, with a $C_{\text{min}}/\text{MIC}$ ratio of 2.06. The regimen with 50% greater exposure produced a $C_{\text{min}}/\text{MIC}$ ratio of 3.09 and resulted in success. For levofloxacin monotherapy, the dose of 1,000 mg daily gave a free drug AUC/MIC ratio of 100, with resistance emergence, while the exposure equivalent to 1,250 mg daily provided a free drug AUC/MIC ratio of 120, with resistance suppression. For combination therapy, the lowest exposures evaluated were 1-h infusions of 1,000 mg every 8 h (for meropenem) and 750 mg.

FIG. 3. MexAB pump-overexpressed PAO1 resistant populations seen in the no-treatment control (A) and the monotherapy regimens with levofloxacin (B to D) and meropenem (E to G).
daily (for levofloxacin). These produced a free drug $C_{\text{min}}$/MIC ratio of 0.92 for meropenem and a free drug AUC/MIC ratio of 62 for levofloxacin, resulting in rapid cell kill and resistance suppression. The combination allowed a 2/3 reduction in the β-lactam and almost a 50% reduction in fluoroquinolone exposure to provide the same result.

For the MexAB pump-overexpressed isolate, this pattern was recapitulated. Combination therapy suppressed resistance at about 40% of the free drug $C_{\text{min}}$/MIC ratio for meropenem, along with a low free drug AUC/MIC ratio of 32 for levofloxacin. It should be noted that the largest levofloxacin exposure examined as monotherapy (levofloxacin at 1,500 mg q24h) produced a free drug AUC/MIC ratio almost twice as high but still allowed resistance emergence. Further, the free drug $C_{\text{min}}$/MIC ratio for meropenem monotherapy associated with success in the MexAB pump-overexpressed isolate was about the same as that associated with failure in the WT isolate (2.06, failure in the WT isolate; 1.88, success in the MexAB isolate). The measured mutational frequency to resistance was 1/1.6 $10^7$ for the WT isolate, while it was less than 1/1.05 $10^8$ for the MexAB isolate, suggesting that the small difference in the free drug $C_{\text{min}}$/MIC ratios necessary for resistance suppression with monotherapy may be explained in this way. Nonetheless, it is clear that the combination therapy resulted in the need for much smaller exposures for both agents to achieve the resistance suppression target for both isogenic isolates.

**DISCUSSION**

*Pseudomonas aeruginosa* continues to be a major source of morbidity and mortality, and in recent years, clinicians have all but lost the ability to treat some isolates. For infections such as
ventilator-associated pneumonia, the bacterial burden is often very high, exceeding the inverse of the mutational frequency to resistance for any single drug. The a priori inference is that there will be a substantial subpopulation of organisms that are less susceptible to the single agent. It should come as no surprise that large trials of monotherapy for nosocomial pneumonia have resulted in a high frequency of emergence of resistance to therapy (amplification of resistant subpopulations). In a study by Fink et al. (8), monotherapy with 400 mg of ciprofloxacin given i.v. every 8 h resulted in a 33% emergence of resistance to therapy for nosocomial Pseudomonas pneumonia. The control arm was imipenem at 500 mg given i.v. every 6 h or 1,000 mg given i.v. every 8 h, which resulted in a 50% rate of Pseudomonas resistance to therapy.

Our laboratory has found breakpoint drug exposure values to decrease the likelihood of resistance emergence for P. aeruginosa (10). We tested this for levofloxacin, showing that the use of the highest labeled dose resulted in resistance suppression in only 61% of cases as determined by Monte Carlo simulation. Against this background, we wished to examine combination therapy for P. aeruginosa.

The classical combination for synergistic interaction is a β-lactam and an aminoglycoside. While this is an important combination, some investigators have recommended against the use of such a combination in very sick intensive care unit patients because of the possibility of nephrotoxicity (17).

As described above, we chose to look at the somewhat unusual combination of a β-lactam and a fluoroquinolone because of the unexpected data reported in a study by West et al. (18). The classical thinking about combinations is not to use combinations of agents that share resistance mechanisms because the orthogonality of resistance probabilities will not hold and one might not expect resistance suppression, as one sees, for example, with tuberculosis (14). Both meropenem and levofloxacin share the Mex efflux pump resistance mechanisms, and it is clear that MexAB overexpression affects both agents, as both MIC values for the isogenic mutant rise relative to the baseline levels for the WT strain. Of interest, the meropenem MIC rises more than the levofloxacin MIC. This is likely because although the MexAB pump expels levofloxacin, the actual preferred pump for levofloxacin is MexCD, as we have shown previously (10).

Structural knowledge of RND pumps, as shown by Murakami et al. (11), demonstrates quite clearly that we can expect that the pump will behave in a Michaelis-Menten fashion. The RND pumps have an access point in the cytoplasm, which is used by the fluoroquinolones, as they penetrate well into microorganisms to gain access to their target site. In contrast, β-lactams have their target site in the periplasm (β-lactam binding proteins), and there is an access point for the pump there. Since the β-lactams do not penetrate well across the inner membrane, the different entry points provide a structural reason why the two drugs together can saturate the pump well. Consequently, from a theoretical standpoint, such a combination is reasonable for evaluation.

In the monotherapy evaluations, either all regimens failed or
TABLE 1. Pharmacodynamic parameters for failing and succeeding regimens of monotherapy and combination therapy

| Isolate and regimen | Free drug $C_{\text{min}}$/MIC ratio for meropenem | Free drug AUC/MIC ratio for levofloxacin | Result based on resistance suppression |
|---------------------|-------------------------------------------------|------------------------------------------|--------------------------------------|
| PAO1 wild type      |                                                 |                                          |                                      |
| M1000q8h†           | 1.42                                            | Failure                                  |                                      |
| M2000q8h†           | 2.06                                            | Failure                                  |                                      |
| M3000q8h†           | 3.09                                            | Success                                  |                                      |
| L750q24h†           | 76.0                                            | Failure                                  |                                      |
| L1000q24h†          | 100.2                                           | Failure                                  |                                      |
| L1250q24h†          | 120.4                                           | Success                                  |                                      |
| M1000q8h† and L750q24h† | 0.92                          | 62.4                        | Success                             |
| MexAB pump-overexpressed mutant |                                           |                                          |                                      |
| M1000q8h*           | 0.554                                           | Failure                                  |                                      |
| M2000q8h*           | 1.47                                            | Failure                                  |                                      |
| M3000q8h*           | 1.88                                            | Success                                  |                                      |
| L750q24h†           | 36.5                                            | Failure                                  |                                      |
| L1000q24h†          | 45.2                                            | Failure                                  |                                      |
| L1500q24h†          | 58.5                                            | Failure                                  |                                      |
| M1000q8h† and L750q24h† | 0.822                          | 31.5                        | Success                             |

* Regimens are identified by the agent (M, meropenem; L, levofloxacin), the dose in milligrams, and the dosage frequency, e.g., M1000q8h indicates meropenem at a dose of 1,000 mg given q8h. †, 1-h infusion.

all but the highest-exposure regimen failed. Failure was due to the emergence of resistance for both the WT and the MexAB pump-overexpressed isolate (Fig. 1, 2, and 3). In contradistinction, all combination therapy regimens for both isolates succeeded, and emergence of resistance was not seen (Fig. 4).

The evaluation of monotherapy and combination therapy by Kaplan-Meier analysis demonstrated a highly significant difference in favor of combination therapy (Fig. 5B and 6B), which can be thought of as synergy for resistance suppression.

Synergistic interaction is most often considered with regard to cell kill. When we examined cell kill as an endpoint, we again found a significant difference between monotherapy and combination therapy (Fig. 5A and 6A). We can thus think of this combination as also being synergistic for cell kill. Many of the combination regimens achieved a 3-log-CFU/ml cell kill at 0.2 days after therapy initiation, and all achieved this by 1 day (Fig. 4). The ability to rapidly decrease the total-population burden is important. Our laboratory has shown previously that the ability of granulocytes to kill Pseudomonas aeruginosa is saturable (6). In that evaluation, the number of organisms that half saturated the granulocyte kill rate was 4.3 $\times$ 10^7. With a 3-log-CFU/ml cell kill occurring quickly after initiation of therapy and without resistance amplification, even quite dense initial bacterial burdens can be reduced to below the saturation point and granulocytes can then contribute significantly to the final eradication of the organisms.

Quantitatively, we can see from Table 1 that both the free drug $C_{\text{min}}$/MIC ratio and the free drug AUC/MIC ratio required for successful suppression of resistant subpopulations were markedly reduced in combination therapy. Generally, the actual required exposures were reduced by factors of 2 to 3. This has an impact on the ability of specific regimens to achieve the necessary targets in combination.

The combination of meropenem and levofloxacin is an appealing combination; each drug used separately has an excellent safety profile, as demonstrated in the clinic, and the combination produces a significantly higher kill rate and significantly better resistance suppression than either agent used as monotherapy, even at exposures that significantly exceed the licensed doses for both drugs. This combination should be evaluated in a randomized clinical trial. It will be optimal to explore lower doses of these agents to identify the lower bounds of free drug $C_{\text{min}}$/MIC ratio and free drug AUC/MIC ratio that will suppress resistance.

ACKNOWLEDGMENTS

This work was supported by grant R01AI079578 from the NIAID to the Emerging Infections and Pharmacodynamics Laboratory, Ordway Research Institute.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health. We have no conflicts to disclose.

REFERENCES

1. Bilello, J. A., G. Bauer, M. N. Dudley, G. A. Cole, and G. L. Drusano. 1994. Effect of 2,3-didehydro-3-deoxythymidine in an intravitro hollow-fiber pharmacodynamic model system correlates with results of dose-ranging clinical studies. Antimicrob. Agents Chemother. 38:1386–1391.
2. Blaser, J. 1985. In vitro model for simultaneous simulation of the serum kinetics of two drugs with different half-lives. J. Antimicrob. Chemother. 15(Suppl. A):125–130.
3. Blaser, J., B. J. Stone, and S. H. Zinner. 1985. Two compartment kinetic model with multiple artificial capillary units. J. Antimicrob. Chemother. 15(Suppl. A):131–137.
4. Clinical and Laboratory Standards Institute. 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. CLSI publication M7-A7. CLSI, Wayne, PA.
5. D’Argenio, D. Z., and A. Schumitzky. 1997. ADAPT II. A program for simulation, identification, and optimal experimental design. User manual. Biomedical Simulations Resource, University of Southern California, Los Angeles, CA. http://bmsr.usc.edu/.
6. Drusano, G. L., C. Fregueau, W. Liu, D. L. Brown, and A. Louie. 2008. Impact of granulocytes on clearance of infection in a mouse thigh infection model: Pseudomonas aeruginosa is different from Staphylococcus aureus, abstr. A-992. Abstr. 48th Annu. Intersci. Conf. Antimicrob. Agents Chemother. (ICAAcT)-Infec. Dis. Soc. Am. (IDSA) 48th Annu. Meet. American Society for Microbiology and Infectious Diseases Society of America, Washington, DC.
7. Drusano, G. L., W. Liu, C. Fregueau, R. Kulawy, and A. Louie. 2009. Differential effects of combination chemotherapy with meropenem and tobramycin on cell kill and suppression of resistance on wild-type Pseudomonas aeruginosa PAO1 and its isogenic MexAB efflux pump-overexpressed mutant. Antimicrob. Agents Chemother. 53:2266–2273.
8. Fink, M. P., D. R. Snyderman, M. S. Niederman, K. V. Leeper, Jr., R. H. Johnson, S. O. Heard, R. G. Wunderink, J. W. Caldwell, J. J. Schentag, and G. A. Slami. 1994. Treatment of severe pneumonia in hospitalized patients: results of a multicenter, randomized, double-blind trial comparing intravenous ciprofloxacin with imipenem-clastatin. The Severe Pneumonia Study Group. Antimicrob. Agents Chemother. 38:547–557.
9. Gumbo, T., A. Louie, M. R. Deziel, L. M. Parsons, M. Salfinger, and G. L. Drusano. 2004. Selection of a mutofloxacin dose that suppresses Mycobacterium tuberculosis resistance using an in vitro pharmacodynamic infection model and mathematical modeling. J. Infect. Dis. 190:1642–1651.
10. Jumbe, N., A. Louie, R. Leary, W. Liu, M. R. Deziel, V. H. Tam, R. Bachhawat, C. Freeman, J. B. Kahn, K. Bush, M. N. Dudley, M. H. Miller, and G. L. Drusano. 2003. Application of a mathematical model to prevent in-vivo amplification of antibiotic-resistant bacterial populations during therapy. J. Clin. Invest. 112:275–285.
11. Murakami, S., R. Nakashima, E. Yamashita, T. Matsumoto, and A. Yamaguchi. 2006. Crystal structures of a multidrug transporter reveal a functionally rotating mechanism. Nature 443:173–179.
12. Pelosiquin, C. A., T. J. Cumbo, D. E. Nix, M. F. Sands, and J. J. Schentag. 1899. Evaluation of intravenous ciprofloxacin in patients with nosocomial lower respiratory tract infections: impact of plasma concentration and clinical condition on bacterial eradication. Arch. Intern. Med. 149:2269–2273.
13. Schimpff, S., W. Satterlee, V. M. Young, and A. Serpick. 1971. Empiric therapy with carbenicillin and gentamicin for febrile patients with cancer and granulocytopenia. N. Engl. J. Med. 284:1061–1065.

14. Selkon, J. B., S. Devadatta, K. G. Kulkarni, D. A. Mitchison, A. S. Narayana, C. N. Nair, and K. Ramachandran. 1964. The emergence of isoniazid-resistant cultures in patients with pulmonary tuberculosis during treatment with isoniazid alone or isoniazid plus PAS. Bull. World Health Organ. 31:273–294.

15. Tam, V. H., A. Louie, M. R. Deziel, W. Liu, and G. L. Drusano. 2007. The relationship between quinolone exposures and resistance amplification is characterized by an inverted U: a new paradigm for optimizing pharmacodynamics to counterselect resistance. Antimicrob. Agents Chemother. 51:744–747.

16. Tam, V. H., A. Louie, T. R. Frütsche, M. Deziel, W. Liu, D. L. Brown, L. Deshpande, R. Leary, R. N. Jones, and G. L. Drusano. 2007. Drug exposure intensity and duration of therapy's impact on emergence of resistance of Staphylococcus aureus to a quinolone antimicrobial. J. Infect. Dis. 195:1818–1827.

17. Uchino, S., J. A. Kellum, R. Bellomo, G. S. Doig, H. Morimatsu, S. Morgera, M. Schetz, I. Tan, C. Bouman, E. Macedo, N. Gibney, A. Tolwany, and C. Ronco. 2005. Acute renal failure in critically ill patients: a multinational, multicenter study. Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) Investigators. JAMA 294:813–818.

18. West, M., B. R. Boulanger, C. Fogarty, A. Tennenberg, B. Weisinger, M. Oross, S.-C. Wu, and J. B. Kahn. 2003. Levofloxacin versus imipenem/cilastatin followed by ciprofloxacin in the treatment of nosocomial pneumonia: a prospective, randomized, multicenter study. Clin. Ther. 25:486–506.