

Pharmacokinetics and Pharmacodynamics of Antimicrobials

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Antibiotics are some of our most commonly used drugs. Until recently, little has been known about how to optimize administration of these agents. Unfortunately, the rate of discovery of new antibiotics has been declining, coincident with the explosion in the number of multidrug-resistant organisms in both the community and hospital environments. This development makes the identification of optimal regimens that will result in good clinical and microbiological outcomes important, but it also makes clear the necessity of identifying regimens that will suppress the emergence of resistant organisms. Given that new agents for multidrug-resistant pathogens will take nearly a decade to become available to physicians, keeping organisms susceptible to drugs that are already available is even more critical. Pharmacodynamics allows identification of the drug exposure measure that is closely associated with the ability to kill organisms and, also, to suppress the emergence of resistant subpopulations of organisms. Use of Monte Carlo simulation allows identification of drug doses in the clinical arena to accomplish these ends. Such approaches should be applied to all old and new antibacterial agents.

Although Dr. Theodore E. Woodward is best remembered for his work in the field of rickettsial diseases, he played a seminal role in the early development of antimicrobial chemotherapy [1–5]. Dr. Woodward was the chairman of the Department of Medicine when I entered the University of Maryland School of Medicine in Baltimore. After medical school and residency, I received a Fellowship in Infectious Diseases. In between, I served a year as chief medical resident, during which time I spent 6 months at the Baltimore Veterans Affairs Medical Center with Dr. Frank Calia and 6 months at the University of Maryland Hospital with Dr. Woodward. During this latter period, a patient was admitted to the intensive care unit with *Klebsiella pneumoniae* pneumonia. As with any “great case,” I and the other residents would seek the wise counsel of Dr. Woodward. I ran up to him in the hallway, presented the case quickly, and said “Dr. Woodward, we’re treating the

patient with cefazolin plus gentamicin. How much should we give him and for how long?” After about 5 seconds of consideration, Dr. Woodward said “George, you treat him with enough and you treat him for long enough!” Thus was born my interest in optimizing the chemotherapy used to treat infectious diseases.

DIFFERENT DRUG CLASSES

The first important issue in the use of chemotherapy for infectious diseases is that drug classes behave differently toward the pathogens at which they are directed. Some drug classes, such as β -lactams (e.g., penicillins, cephalosporins, monobactams, and carbapenems), have their rate of killing maximized at a low multiple of the MIC. Achieving higher drug concentrations does not result in greater cell killing. The reason for this finding is because, as demonstrated by Williamson and Tomasz [6], little happens to the organism physiologically until a considerable portion of the β -lactam-binding proteins are occupied. As the drug concentration increases, the effect quickly maximizes. Consequently, the maximum effect is achieved at a low multiple of the MIC [7]. For β -lactam agents,

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Clinical Infectious Diseases 2007;45:S89–95

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1058-4838/2007/4502S1-0019\$15.00

DOI: 10.1086/518137

the best therapeutic results are obtained by using smaller doses more frequently for any daily dose.

The association between the time that free drug concentrations remain in excess of the MIC (time > MIC) and the ability of the regimen to kill organisms at the site of infection was first demonstrated by Eagle et al. [8], in a mouse thigh infection model of *Streptococcus pneumoniae* infection and penicillin therapy. Four decades later, Craig [9] elegantly demonstrated the same finding in a mouse model of *K. pneumoniae* pneumonia, with the cephalosporin ceftazidime used as the study agent (figure 1). Time > MIC is far more explanatory of cell killing for this agent than are other measures of drug exposure, such as the peak concentration:MIC ratio or the area under the concentration-time curve (AUC):MIC ratio.

When one examines other drug classes (e.g., aminoglycosides or fluoroquinolones), different measures of exposure, such as the AUC:MIC ratio or the peak concentration:MIC ratio, are better at explaining the amount of microbiological activity seen with different exposures. Our group of investigators demonstrated this for the fluoroquinolone lomefloxacin in a rat model of *Pseudomonas sepsis* [10]. This animal model differs from others in the very large inoculum used intraperitoneally ($\sim 10^9$ organisms). This number exceeds the inverse of the frequency of resistance mutations for the challenge isolate; a resistant subpopulation is present a priori. When a large dose (80 mg/kg) is administered once daily, as one-half of the dose every 12 h, or as one-fourth of the dose every 6 h, the single large dose is significantly better at preventing death (figure 2A). This finding would indicate that the peak concentration:MIC ratio is associated with outcome. However, when the dose is reduced to 40 mg/kg and is fractionated as a once-daily dose or as 20 mg/kg every 12 h (figure 2B), no difference in survival is seen; this finding implies that the AUC:MIC ratio is most

closely associated with outcome. It is highly likely that confounding of an end point of organism kill is associated with the suppression of resistance and the ultimate effect on survival. In figure 2A, the large dose likely has some effect (although suboptimal, given the incomplete survival noted) on the resistant mutant subpopulation. Only the single daily dose would achieve a concentration that would have any effect at all on the resistant mutants that make up a small part of the initial challenge population. In figure 2A, both fractionated regimens are equivalent in terms of effect, and, in figure 2B, the lower-dose regimens are equivalent in terms of effect, irrespective of the schedule of administration. If a smaller inoculum and a different pathogen and model (e.g., *S. pneumoniae* and mouse thigh infection model) are used, the AUC:MIC ratio again appears to be the most explanatory exposure variable (figure 2C) [11]. More will be discussed regarding this issue in the section on suppressing the emergence of resistance by dosing.

Finally, all exposure measures (peak concentration:MIC ratio, AUC:MIC ratio, and time > MIC) have a component of drug concentration and an MIC measurement. The question arises as to which is most important. In figure 2D, this question is addressed. Three isogenic strains were created from the original *Pseudomonas* challenge isolate, with MICs of fluoroquinolone of 1 mg/L, 4 mg/L, and 8 mg/L. A cohort of rats was infected with each strain and was treated with the same dosage (80 mg/kg/day). Control cohorts are not shown, for reasons of clarity, but all untreated rats died. Survival rates were 65% for rats infected with the strain that had an MIC of 1 mg/L, 15% for those infected with the strain that had an MIC of 4 mg/L, and 0% for those with infected with the strain that had an MIC of 8 mg/L. In addition, a fourth cohort was infected with the isolate for which the MIC was 1 mg/L and was treated with 20 mg/kg/day, resulting in the same peak concentration:MIC ratio

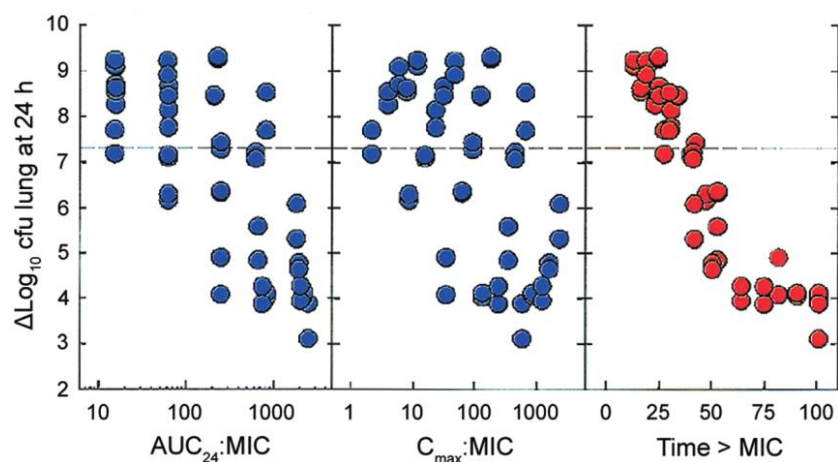


Figure 1. Relationship between different measures of drug exposure and the microbiological effect observed in a mouse model of pneumonia. Murine *Klebsiella pneumoniae* pneumonia was treated with different doses and schedules of ceftazidime. AUC₂₄, area under the 24-h concentration-time curve; C_{max}, maximum concentration. Data are from [9].

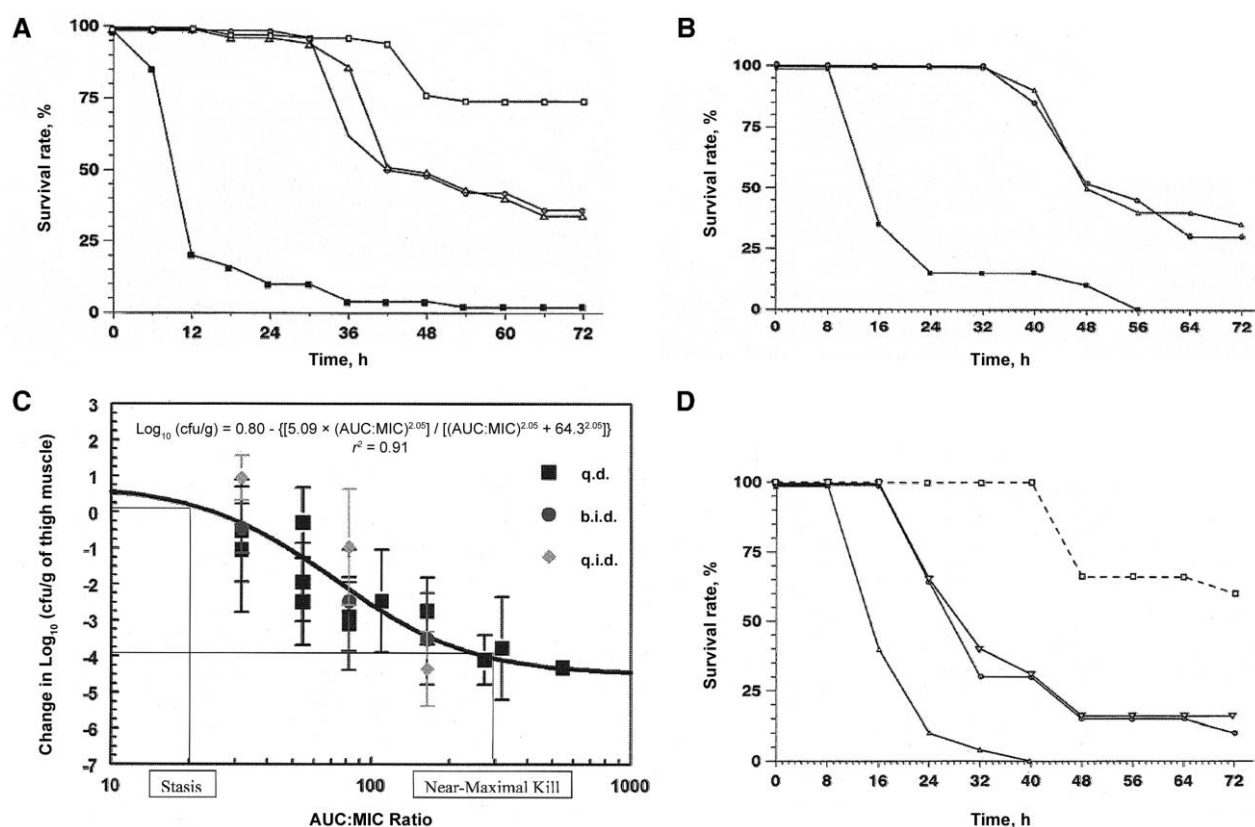


Figure 2. A, Dose fractionation experiment 1. The MIC of lomefloxacin for the challenge organism was 1 mg/L. Regimens of 80 mg/kg every 24 h (□), 40 mg/kg every 12 h (△), and 20 mg/kg every 6 h (●) were evaluated. Control rats received a saline placebo injection (■). Fifty rats were evaluated per group. B, Dose fractionation experiment 2. The MIC of lomefloxacin for the challenge organism was 1 mg/L. Regimens of 40 mg/kg every 24 h (△) and 20 mg/kg every 12 h (●) were evaluated. Control rats received a saline placebo injection (■). Twenty rats were evaluated per group. C, Dose fractionation experiments (q.d. denotes that the whole dose was given once; b.i.d., that one-half the dose was given every 12 h; and q.i.d., that one-fourth of the dose was given every 6 h). The data are displayed with the area under the concentration-time curve (AUC):MIC ratio as the independent variable. The peak concentration:MIC ratio and the time that free drug concentrations remain in excess of the MIC (time > MIC) are also evaluated as independent variables, but the AUC:MIC displayed the best fit of the model to the data. The inoculum was $6.5 \log_{10}$ cfu/thigh. When tested by analysis of variance, no difference was seen between administration of the same total daily dose every 24 h (the whole dose given once), every 12 h (one-half of the dose given every 12 h), and every 6 h (one-fourth of the dose given every 6 h). D, Treatment of groups ($n = 20$) of neutropenic rats infected with 10^3 cfu/mL of 3 isogenic strains of *Pseudomonas aeruginosa* with a fluoroquinolone (lomefloxacin). One group (□) was infected with the parent strain, for which the fluoroquinolone MIC was 1.0 mg/L, and was treated with 80 mg/kg once daily. Another group (▽) was infected with a daughter strain, for which the fluoroquinolone MIC was 4.0 mg/L, and was treated with 80 mg/kg once daily. A third group (△) was infected with a second daughter strain, for which the fluoroquinolone MIC was 8.0 mg/L, and was treated with 80 mg/kg. The final group displayed (●) was infected with the parent strain (MIC, 1.0 mg/L) but was treated with 20 mg/kg. This provides the same AUC:MIC ratio as does being infected with a strain for which the MIC is 4.0 mg/L and being treated with 80 mg/kg. As the MIC increases, with treatment staying constant at 80 mg/kg, the survival rate decreases. When different doses are used but the same AUC:MIC ratios result, the survival rates are not significantly different. Adapted with permission from [10] (A, B, and D) and [11] (C).

and AUC:MIC ratio that were noted for rats infected with the isolate for which the MIC was 4 mg/L and treated with 80 mg/kg/day. These 2 cohorts had identical survival curves. Clearly, the MIC matters. It is more difficult to treat infections for which the organism has a higher MIC. Dose also matters. One-fourth of the dosage (20 mg/kg/day) produced a survival rate of 15% relative to the survival rate (65%) noted when 80 mg/kg/day was given. However, of greater significance is the finding that the ratio of drug exposure to the MIC is what truly drives outcome. The 20 mg/kg/day dosage used for the cohort infected

with the isolate for which the MIC was 1 mg/L had the same peak concentration:MIC ratio and AUC:MIC ratio as did the cohort that was treated with 80 mg/kg/day after being infected with a pathogen for which the MIC was 4 mg/L, and overlapping survival curves were noted.

DEFINING EFFECT TARGETS

It is important to recognize that differing amounts of drug exposure will translate into differing degrees of antimicrobial

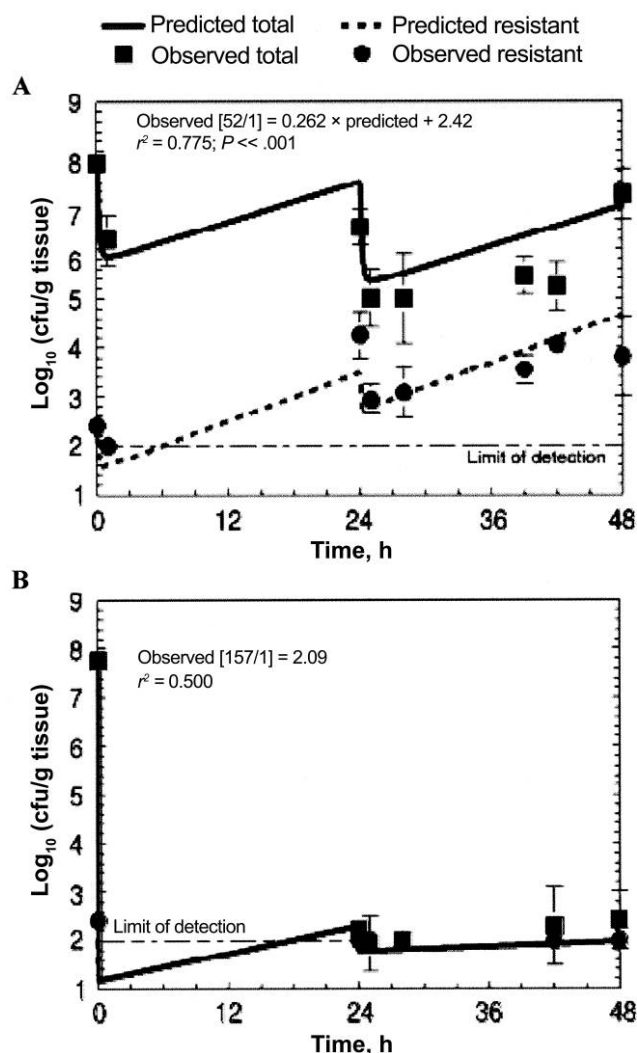


Figure 3. Model validation. The emergence-of-resistance model developed was prospectively validated by generating response predictions for doses not previously studied that would (1) encourage selection of resistance or (2) suppress emergence of resistance. An exposure of an area under the concentration-time curve (AUC):MIC ratio of 157 was calculated to prevent emergence of resistance. Experiments were performed to 48 h, not to 24 h (as in the studies performed to generate parameter estimates). Levofloxacin dosing occurred at time 0 and at 24 h. The lines are model predictions (not best-fit curves). The model predicted changes in the resistant mutant population well at both exposures. Adapted with permission from [13].

effect. For β -lactam agents, this means that the whole dosing interval need *not* be covered by free drug concentrations in excess of the MIC to obtain maximal organism killing at the primary infection site. Examination of figure 1 reveals that ceftazidime concentrations need to exceed the MIC for ~65% of the dosing interval to achieve maximal cell killing. For cephalosporins, stasis occurs at ~35%–40% of the dosing interval covered by free drug. For carbapenems, stasis and near-maximal

cell killing occur at 20% and 40% of the dosing interval, respectively. For penicillins, these end points occur at 30% and 50% of the dosing interval [7].

Examination of figure 2C reveals that, for fluoroquinolones, the AUC:MIC ratio is most closely associated with outcome and also clearly demonstrates that stasis occurs at an AUC:MIC ratio of 20–25 and achieves maximal cell killing at an AUC:MIC ratio of ~250–300.

What yet remains to be well defined is the degree of microbiological effect that correlates with a good clinical or microbiological outcome. Some data do exist with respect to this question. Ambrose et al. [12] have shown that, for community-acquired pneumococcal pneumonia, a good clinical outcome occurs at an AUC:MIC ratio of ~30, indicating that

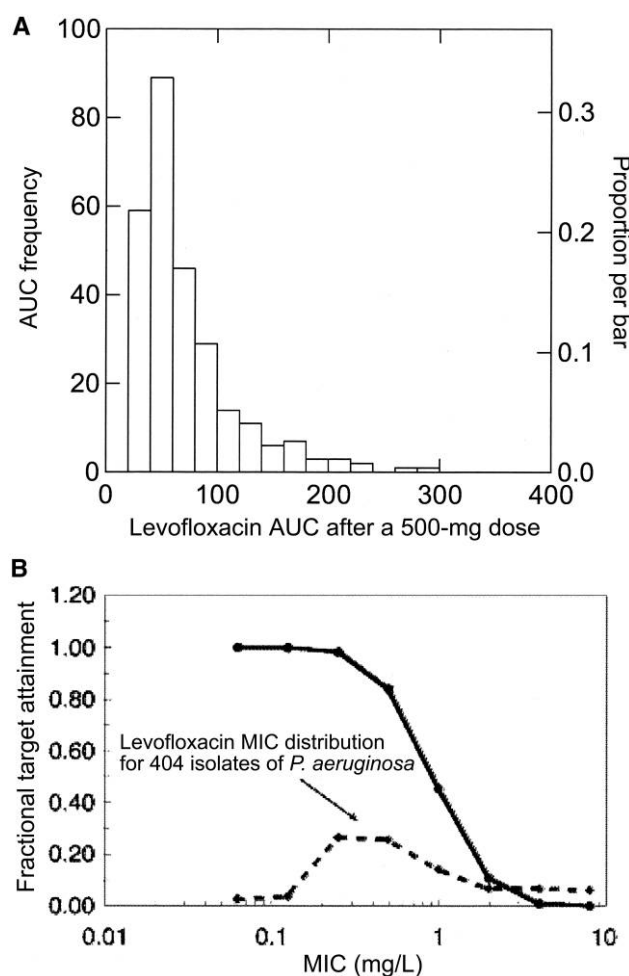


Figure 4. A, Area under the concentration-time curve (AUC) distribution from a study of the use of levofloxacin in 252 patients with community-acquired infection. Data are from [16]. B, Target-attainment analysis. The fraction of 10,000 simulated subjects that attained an AUC:MIC ratio of 157:1 (target for suppression of resistance) is displayed as a function of the MIC for a distribution of 404 isolates of *Pseudomonas aeruginosa*. Adapted with permission from [13].

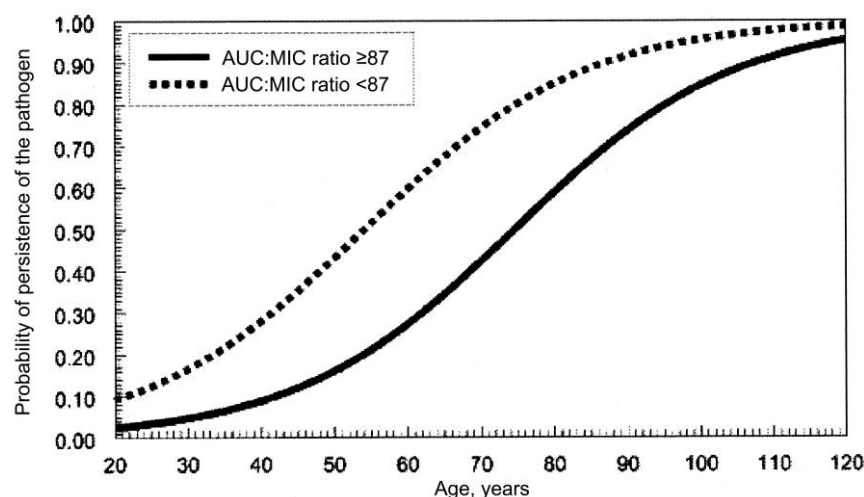


Figure 5. Probability of eradication of the pathogen, as a function of age and whether the patient achieves an area under the concentration-time curve (AUC):MIC ratio ≥ 87 . Younger patients who achieve an AUC:MIC ratio ≥ 87 have a significantly higher probability of achieving eradication of the infecting pathogen. Classification and regression tree analysis identified the breakpoint for age as 67 years. Adapted with permission from [14].

slightly more than stasis is required for a good clinical outcome for this condition, which was defined as a Fine score of 1 or 2 for community-acquired pneumonia in most patients. In another study, our group demonstrated that a decrease in *Pseudomonas aeruginosa* organisms of 2 \log_{10} (cfu/g) occurred in a mouse thigh infection model at an AUC:MIC ratio of 88 [13]. A clinical study of nosocomial pneumonia that was performed using the same fluoroquinolone (i.e., levofloxacin) demonstrated that an AUC:MIC ratio of 87 correlated with a significantly higher probability of a good microbiological outcome [14].

ANOTHER DRUG EXPOSURE TARGET— SUPPRESSION OF RESISTANT SUBPOPULATIONS

In infections such as ventilator-associated pneumonia, the burden of microorganisms can become quite large and frequently exceeds the inverse of the frequency of resistance mutations. This finding implies that there is a small population of resistant organisms that will be present (with a high probability) at the time that therapy is initiated. A drug exposure that will adequately kill the susceptible population may allow amplification of the less-susceptible subpopulation, resulting in emergence of resistance during therapy.

Our group of investigators examined this issue in a mouse thigh infection model [13]. Five parallel inhomogenous differential equations described the drug concentrations after different drug doses, as well as the impact of the drug exposure on the susceptible and less-susceptible bacterial populations over time. We were able to calculate 2 drug doses: (1) a dose

that would maximally amplify the less-susceptible bacterial population (AUC:MIC ratio, 52) and (2) a dose that would prevent the amplification of this subpopulation (AUC:MIC ratio, 157). A prospective validation was performed using these calculated doses. In figure 3, the lines are not best-fit lines but, rather, prediction lines, about which the observations are scattered. As can be seen, the suboptimal dose kills $\sim 0.5 \log_{10}$ (cfu/g) of the total population, but it actually amplifies the resistant subpopulation by $\sim 1.5 \log_{10}$ (cfu/g), indicating that this regimen will trade susceptible clones for less-susceptible clones. The dose predicted to prevent resistance does indeed suppress the less-susceptible subpopulation. To our knowledge, this is the first prospective validation experiment to demonstrate a drug dose and schedule of administration that will suppress resistance emergence. We were able to recapitulate this approach in an in vitro hollow fiber infection model [15]. Given the lack of new agents with gram-negative activity on the near-term (7- to 10-year) horizon, maintaining drug susceptibility through proper dosing would seem to be prudent.

DEFINING THE DRUG DOSE THAT WILL RESULT IN THE DESIRED EFFECT

Once we have a goal of therapy, it is important to define the drug dose that will attain the desired target with a high probability. Giving the same drug dose to a large number of patients will result in a wide range of exposures. We studied 272 patients with community-acquired infections treated with levofloxacin [16]. The range of AUC values achieved was quite large (figure 4A). It is also important to recognize that target pathogens also have a range of MICs. As is demonstrated in figure 2D, higher

MICs have an impact on outcome. When choosing a drug dose to attain the exposure target, it is important to recognize the range of MICs that might be encountered clinically and the percentage of a large collection of isolates at each MIC in the distribution.

To evaluate a specific drug dose, we need the protein binding of the drug, because only free drug is microbiologically active [17]. To describe the likely range of exposures that will occur clinically, previous information regarding the central tendency and measure of dispersion of the pharmacokinetic parameter values is required. We can then use Monte Carlo simulation to calculate the proportion of patients obtaining a specific degree of drug exposure. The exposures are then corrected for protein binding. Afterward, the fraction of simulated subjects who attained the exposure target (e.g., AUC:MIC ratio or time > MIC) intended is then calculated for each MIC in the distribution. The overall target attainment is then calculated by taking the product of the target attainment at a specific MIC and the fraction of organisms in the distribution at that MIC. All products are then summed, giving a weighted average target attainment rate that takes into account the variability in MICs as well as the variability in pharmacokinetic parameter values across a population of patients. An example is provided in figure 4B. In that figure, the target is for suppression of resistance (AUC:MIC ratio, 157), and a Monte Carlo simulation was performed using pharmacokinetic data from a clinical trial. A population of *P. aeruginosa* had an MIC of levofloxacin determined ($n = 404$), and the target attainment rates are presented according to the MIC. The overall target attainment rate was 62%, which indicates that, at a dose of 750 mg, it would be wise to add a second agent to levofloxacin therapy to suppress resistant mutants of this pathogen. Our laboratory first described this technique of setting breakpoints and evaluating drug doses at a meeting of the Anti-infective Drug Products Advisory Committee [18] and later published a description of the technique [19].

PHARMACOKINETICS/PHARMACODYNAMICS IN CLINICAL TRIALS

Although it is important to address the association between exposure and response preclinically, it is also vitally important to define the association in real patients. Studying hospitalized patients is difficult because of the disease process present and because of the limitation on obtaining blood. Our group of investigators validated optimal sampling theory in a number of studies performed at the University of Maryland in the late 1980s and beyond [20–23]. These validations allow identification of information-rich sampling times, so that small numbers of blood draws can still provide robust pharmacokinetic information for individual patients. We also examined combining optimal sampling theory with population pharmaco-

kinetic modeling [24]. Once the population pharmacokinetic model is fit to the data, estimates can be obtained for individual patients through Bayesian estimation. If one then knows the infecting pathogen and the MIC for the drug, as well as the clinical/microbiological outcome, the drug exposure (e.g., AUC:MIC ratio or time > MIC or peak concentration:MIC ratio) can be associated with the outcome by use of logistic regression analysis. For time-to-event end points, such as time to defervescence and time to death, a Cox proportional hazards model would be employed.

A series of mathematical techniques can be used in concert to allow the modeling of patient outcome as a function of measures of drug exposure and other patient-driven covariates, such as demographics, Acute Physiology and Chronic Health Evaluation II score, and other such measures. These techniques include (1) optimal sampling theory, to guide blood draw times; (2) population pharmacokinetic modeling, to allow generation of central tendencies and dispersions for population pharmacokinetic parameter values; (3) Bayesian estimation, to bring drug exposure back to the individual patient; and then (4) linkage of exposure to response, by use of MIC, as well as outcome and other patient covariates.

Forrest et al. [25] published the first retrospective evaluation of this type for the fluoroquinolone ciprofloxacin. Our group later published what is the first fully prospective study of this type [16] involving community-acquired infections treated with levofloxacin. Ambrose et al. [12] investigated the use of gatifloxacin and levofloxacin for community-acquired pneumococcal pneumonia. Subsequently, we examined the β -lactam cefepime [26]; aminoglycosides, for both efficacy and toxicity [27, 28]; and, most recently, patients with nosocomial pneumonia treated with levofloxacin [14]. In this latter study, we were able to identify a breakpoint value in the AUC:MIC ratio of 87 as denoting a significantly improved probability of attaining a good microbiological outcome. After examination of 18 covariates, only 2 factors were included in the final model: (1) whether the AUC:MIC ratio was achieved and (2) the age of the patient (older age was not beneficial). The outcome for the model is presented in figure 5.

Clearly, drug exposure plays a key role in determining the outcome for seriously infected patients. This area of inquiry is one of the most rapidly growing areas in infectious diseases. I like to think that Dr. Woodward would smile at that and think of that patient with *K. pneumoniae* pneumonia.

Acknowledgments

There are many people at the University of Maryland School of Medicine who deserve thanks. Some, but not all, are Richard Hornick, Stephen Schimpff, Jack Warren, Dave Johnson, and, most especially, Merrill Snyder, Frank Calia, and Harold Standiford.

Supplement sponsorship. This article was published as part of a supplement entitled "Tribute to Ted Woodward," sponsored by an unrestricted

grant from Cubist Pharmaceuticals and a donation from John G. McCormick of McCormick & Company, Hunt Valley, Maryland.

Potential conflicts of interest. G.L.D.: no conflicts.

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