

Integretion of pharmacokinetics and pharmacodynamics in antibacterial drug development and pharmacotherapy

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ABSTRACT There is a pressing need for new antibacterial agents due to the development of drug-resistant pathogens. Unfortunately drug development is a difficult and complicated process. The traditional approach in searching for a right dose is quite empirical, both costly and time-consuming. To enhance the ability to predict the likelihood of success for lead compound selection, *in vitro* pharmacodynamic and *in vivo* animal infection models are now extensively used. The value of these pre-clinical experiments, combined with mathematical modeling, helps to identify a pharmacokinetic (PK)-pharmacodynamic (PD) exposure measure which best predicts the therapeutic efficacy, and to quantify the magnitude of this index required for *in vivo* efficacy. PK-PD target attainment analyses using Monte Carlo simulation to integrate interpatient variability in drug exposure (PK), drug potency (MIC), and *in vivo* exposure targets that are predictive of positive therapeutic outcomes are influencing antibacterial drug development for proof of concept, for dose and dosing interval selection, for determining susceptibility breakpoints, and for evaluating the clinical meaning of antibacterial resistance. In this article, the key concepts of antibacterial PK-PD and model based antibacterial drug development strategy and process are critically reviewed.

KEY WORDS pharmacokinetics; pharmacodynamics; antibacterial; *in vitro* model; animal models; Monte Carlo simulation; model based drug development

Antibacterial drugs were the most effective of all medicines. The US Surgeon General once said: "The

time has come to close the book on infectious diseases"^[1]. Such optimism has unfortunately proved wrong. Resistance to all major classes of antibiotics is now commonplace which has led to the prediction that we are reentering the pre-antibiotic era. Furthermore, today's pipeline of potential replacement drugs is the weakest ever in the history of antibacterial drug development. Several factors contributed to this paradox: first, many large pharmaceutical companies perceive that development of novel antibacterial drugs is now less profitable than other therapeutic areas; second, for many indications, such as community-acquired respiratory tract infections, there has been considerable regulatory uncertainty about clinical endpoints and statistical criteria needed to show efficacy of experimental drugs; last but not the least, it is technically very challenging to develop a novel antibacterial agent, primarily due to difficulties in dose regimen determination. The success of antibacterial therapy is determined by complex interactions between the administered drug, the host, and the infecting pathogen. In a clinical situation, the complexity of these interactions is usually reflected by a high variability in the dose-response relationship. Therefore, to minimize the dose-response variability, key characteristics of the drug, the infecting agent and the host have to be taken into account for selecting an appropriate antibacterial and an appropriate dose. Inability to do so may result in either therapeutic failure or the emergence of resistant strains. Over the past decade, a number of pharmaco-statistical tools, such as population pharmacokinetic modeling and Monte-Carlo simulation, optimal sampling theory, and Bayesian estimation have become widely available to investigators that allow estimations of PK exposure in individual patients. Integration of pharmacokinetics-pharmacodynamics (PK-PD) in antibac-

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terial drug development allows the drug dose to be chosen in a rational manner. PK-PD target attainment analyses using Monte Carlo simulation to integrate interpatient variability in drug exposure (PK), drug potency (MIC), and *in vivo* exposure targets that are predictive of positive therapeutic outcomes are influencing antibacterial drug development for proof of concept, for dose and dosing interval selection, for determining susceptibility breakpoints, and in evaluating the clinical meaning of antibacterial resistance^[2].

This paper will first review key concepts of antibacterial PK-PD, and then discuss the recently emerged model-based drug development strategy by first introducing *in vitro* PD and *in vivo* animal infection models and their value in the PK and PD characterization of antibacterials; and second, by presenting how to integrate the information obtained in the preclinical stage with the one gathered in clinical stage to come up with the rational dosage regimen strategy for Phase III trials and clinical therapy.

1 BASIC CONCEPTS OF ANTIBACTERIAL PHARMACOKINETICS-PHARMACODYNAMICS

Before introducing integration of PK-PD in antibacterial drug development and therapy, a few relevant concepts need to be discussed first.

1.1 Time-dependent and concentration-dependent killing

Using the minimum inhibitory concentration

(MIC) based PK-PD indices, antibacterials are frequently divided into three major groups: a) those that exhibit concentration-dependent killing and prolonged persistent effects; b) those that exhibit time-dependent killing and minimal persistent effects, and c) those that exhibit time-dependent killing and moderate to prolonged persistent effects.

The most common PK-PD indices are a) the duration of time a drug concentration remains above the MIC ($T > MIC$), it is usually expressed as the ratio of such time duration over the dosing interval ($\%T > MIC$); b) the ratio of the maximal drug concentration to the MIC (C_{max} / MIC); and c) the ratio of the area under the concentration time-curve at 24 h to the MIC (AUC_{24MIC}) (Fig 1). The PK-PD index that is most predictive of efficacy is dependent upon the agent's pattern of bactericidal activity, and the presence and duration of persistent effects (Table 1).

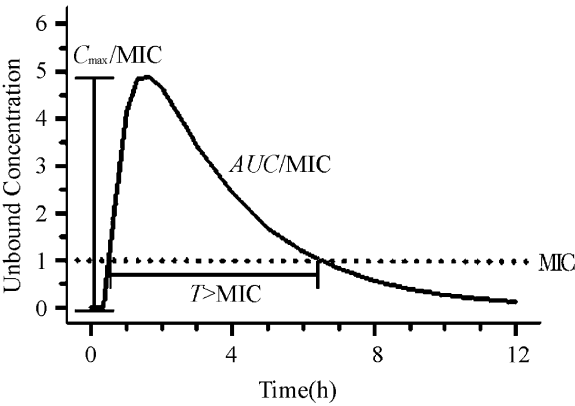


Fig 1 PK-PD indices correlating with antibacterial efficacy

Table 1 Patterns of bacterial activity and PK-PD indices correlating with efficacy

Pattern of Activity		Antibiotics	Goal of Therapy	PK/PD Parameter
Type I Concentration-dependent killing and Prolonged persistent effects		Aminoglycosides	Maximize concentrations	AUC/MIC C_{max} / MIC
		Daptomycin		
		Fluoroquinolones		
		Ketolides		
Type II Time-dependent killing and Minimal persistent effects		Carbapenems	Maximize duration of exposure	$T > MIC$
		Cephalosporins		
		Penicillins		
Type III Time-dependent killing and Moderate to prolonged persistent effects		Macrolides	Maximize amount of drug	AUC/MIC
		Azithromycin		
		Clindamycin		
		Linezolid		
		Tetracyclines		
		Vancomycin		

For the aminoglycosides and fluoroquinolones, bacterial killing is concentration dependent. For these agents, the shape of the concentration-time curve does not have a significant impact on the microbiological effect observed. The higher the drug concentration, the faster and more extensive is the rate of killing. Thus, the peak level (C_{\max}) and the amount of drug, as reflected by the area under the concentration-versus-time curve (AUC) in serum, are important predictors of the efficacy of fluoroquinolones and aminoglycosides. C_{\max}/MIC levels of 8 to 10 are required for high response rates with aminoglycosides. This has led to once-daily dosing of these drugs. This form of dosing can also lessen the frequency or the rate at which nephrotoxicity develops.

Bacterial killing by β -lactam antibiotics, on the other hand, is slower and shows little dependence on the drug concentration. Their effect of β -lactams depends on the length of time that the drug is in contact with the bacteria. Their effect will increase with increasing concentrations until the maximum kill rate is reached. After that point, increasing concentrations will not produce a corresponding increase in the effect; therefore, high peak concentration will not help. Maximum killing has been seen to occur at concentrations approximately four to five times the MIC^[3]. The duration of time that drug concentrations exceed the MIC is the important predictor of efficacy for these drugs.

The presence and duration of post-antibiotic effect is also important to define an agent's bactericidal pattern of activity. There is another group of drugs for which the AUC/MIC ratio is linked to outcome, but these agents are time-dependent in their effect. Examples of this group include drugs such as azithromycin and vancomycin^[4,5]. These agents are either poorly bactericidal or slowly bactericidal. In either cases, these drugs cause a profound post-antibiotic effect (PAE), that is, after exposure to the drug and complete drug removal, the time for which bacteria take to regrow 1 log₁₀ colony-forming unit per milliliter (CFU/mL). This is probably related to the bacterial injury that is caused by the drug exposure. For the agents cited, the duration of the PAE is prolonged by increasing the AUC/MIC ratio. Of interest for these drugs is that as the MIC increases and the AUC/MIC ratio decreases, the duration of the PAE is reduced and these agents become more similar to $T > MIC$ drugs.

Moderate-to-long post-antibiotic effect is the rule, rather than the exception, for gram-positive bacteria like staphylococci^[6]. However, for gram-negative bacteria, a significant post-antibiotic effect is primarily observed with agents that inhibit protein or nucleic acid synthesis, such as chloramphenicol, macrolides, quinolones, rifamycins, and tetracyclines^[7]. β -Lactams, with the exception of carbapenems (primarily against *P. aeruginosa*), have little post-antibiotic effect against gram-negative bacteria^[9].

1.2 Protein binding and infection site concentration

Serum protein binding is an important PK factor that can affect antibacterial efficacy. Protein binding reduces the activity of antibacterials; it is only the free drug fraction that can be distributed to tissue and has pharmacological activity at any point in time. Thus, it is the free peak concentration, the AUC of free drug, and the time above MIC for free drug that are the important determinants of *in vivo* antibacterial activity. Importantly, the use of the prefix *f* is recommended as an indicator that the free, or unbound, fraction is used or meant when using a PK or PK-PD parameter, e. g. $fAUC/MIC$, fC_{\max}/MIC , $fT > MIC$. For certain drugs, protein binding has species dependent variation. Therefore, when comparing PD between species, free drug concentrations should be used. Protein binding can also affect the elimination of some antibacterials. For example, the high protein binding of ceftriaxone and ertapenem slows the filtration of these drugs by the kidney and significantly extends the duration of their elimination half-life^[9,10].

Drug penetration to the infected tissue is another important PK factor affecting efficacy for certain antibacterials. In the past, tissue concentrations are commonly measured in the matrix of tissue homogenates^[11]. However, tissues consist of two separate compartments that are mixed together when tissue homogenates are produced. The interstitial compartment and the intracellular compartment are distinct sites, and drugs often vary in their distribution in these two compartments. For example, β -lactams provide high levels in interstitial fluids that are similar to those in serum, but very low to undetectable levels in intracellular fluid^[11]. On the other hand, fluoroquinolones produce low interstitial and serum concentrations, but very high levels in intracellular fluid^[12]. Since the intracellular compartment is usually of larger volume than

interstitial fluid, tissue homogenates give lower levels than serum for β -lactams and much higher levels than serum for macrolides. The important variable is the location of the pathogen, which for most bacteria is the extracellular space or interstitial fluid. Thus, for most pathogens the unbound serum concentrations provide a good reflection of the interstitial drug levels and can be used for prediction of PK at the site of action^[13, 14].

Clinically the more important consideration is impaired tissue penetration of antibacterials which results in treatment failure^[15]. Impaired tissue penetration is best recognized for central nervous system (CNS) infections^[16]. The barrier mechanism of the CNS and other organs, such as the eye, is the presence of active transport pumps that lead to target site concentrations which, even at equilibrium, are lower than those in plasma^[17]. This mechanism, which has been well described both *in vitro* and *in vivo*, is likely to be the reason for therapeutic failures in CNS and eye infections^[17]. However, besides CNS infections, many other situations in which impaired drug penetration and blood-tissue inequilibrium can be observed. In particular, antibacterial failures have been attributed to impaired target site penetration in cases of osteomyelitis, peridontitis, prostatitis, eye infections, ear infections, and many others^[18]. Attempts have been made to measure the drug exposure in these target tissue fluids, e.g., middle ear fluid, prostate gland fluid and skin blister fluid, etc. Recently, imaging techniques and microdialysis have been developed to study target tissue distribution kinetics. Microdialysis can be used readily and routinely for clinical and preclinical studies in almost any research center^[18].

Investigation of drug exposure in the lungs of humans has been carried out by measuring drug levels in the epithelial lining fluid (ELF) for extracellular respiratory pathogens or in alveolar macrophages containing intracellular respiratory pathogens (*Legionella pneumophila* or *Chlamidia pneumoniae*) by means of bronchoalveolar lavage. Macrolides, ketolides, and azalides, although they do so to different degrees, all attain considerably higher concentrations in ELF or alveolar macrophages relative to plasma. White blood cells act as transporters of these agents to the site of infection and release of significant amounts of drug during phagocytosis^[19]. Although, the mechanism to explain higher than plasma level is unknown, human ELF penetration data should be considered

when deciding the magnitude of PK-PD index required for a successful microbiologic outcome^[19]. Generally speaking, lipophilic agents, such as macrolides, most fluoroquinolones, tetracyclines, chloramphenicol, rifampicin, and oxazolidinones can readily penetrate into tissues producing intracellular accumulation. Therefore, they are active against the intracellular pathogens. Conversely, hydrophilic agents, such as β -lactams, glycopeptides, and aminoglycosides, are unable to passively diffuse through the cell membrane, and thus, they are inactive against the intracellular pathogens.

In summary, data on tissue penetration of drugs could provide important clinical information. The concentration profile at the target site is an important determinant of clinical outcome and is more predictive in this respect than the concentration in plasma^[18].

1.3 PD biomarker, surrogate and clinical endpoints

Antibacterial PD is a discipline that attempts to link measures of drug exposure to some measure of effect. In clinical trials, the microbiological outcome (bacterial eradication or persistence) or clinical outcome (cure or failure) is often used as surrogate endpoint for clinical efficacy. Both of these effect data are dichotomous variables, giving little information on the dynamics of drug action.

One unique advantage of antibacterial PD is that the drug's ability to affect the pathogen can be measured directly via the minimal inhibitory concentration (MIC) for the pathogen in question. MIC is the lowest concentration that results in stasis, representing the potency of the antibacterial agent against a bacterial strain. MIC is often used as a PD biomarker, indicating a pathogen's susceptibility to an antibacterial. The MIC value has a few limitations in that it does not provide information on the rate of bactericidal activity and whether increasing antibacterial concentrations can enhance the kill rate, nor does it provide any information about the persistent activity of the antibacterial agent that remains following exposure to the drug^[15]. In addition, usually only 1 dose and 1 dosing interval are studied in clinical trials, making discrimination of the PK-PD indices impossible. Therefore, preclinical models are often used to provide this information. Both *in vitro* PD and *in vivo* animal infection models provide an assessment on the rate (slope steepness) and extent (change in \log_{10} CFU/mL) of bacterial killing. These

models can detail the time course relationship between drug concentration and antibacterial efficacy. Modeling of such relationships can determine which PK-PD dosing index best correlates with treatment outcomes. By correcting for inter-species PK differences these studies can also determine the magnitude of the PK-PD index necessary for antibacterial efficacy across animal species, including humans. This should not be surprising, as the target of an antibacterial agent is in the pathogen and not in the animal species. These analyses have been shown to be predictive of therapeutic success and failure against resistant microorganisms in clinical trials. The ability to provide a PK-PD index magnitude target for clinical dosing has been particularly valuable for predicting outcomes against pathogens not encountered frequently enough in clinical trials to come to accurate conclusions.

2 MODEL BASED ANTIBACTERIAL DRUG DEVELOPMENT

The high cost and high attrition rates for bringing new medicines to the market demand more efficient and effective drug development strategies. Drug development is a sequential process involving iterative learn and confirm cycles. The strategy of the developmental value chain from discovery to preclinical through Phase I to Phase III and beyond is to develop and utilize new technologies, *in vitro* or animal models, that are less expensive but still predictive of human PK-PD *in vivo*, and to maximize the information gained in human to support the drug label. The integration of PD information using biomarkers, surrogate markers and clinical endpoints from early stage to late stage represents a more efficient and effective drug development paradigm, i.e., model based drug development (MBDD). As identified by the US FDA, MBDD, a mathematical and statistical approach, can guide the decision making process on lead generation, optimization and product realization. In the past decade, this strategy was implemented in antimicrobial drug development which allowed continuous microbiological variable in the preclinical study to enrich the dichotomous microbiological variable in clinical study (eradication vs. persistence). Through the use of Monte Carlo simulation, one can bridge the preclinical to Phase II/III development. Predictions made by the proposed mathematical modeling framework can offer guidance for targeted testing of prom-

ising regimens. This can aid the development and clinical use of antimicrobial agents that combat microbial resistance^[20].

2.1 Preclinical development The major goal of PK-PD studies in preclinical models is to establish the PK-PD target required for effective antibacterial therapy in humans in a cost-effective manner. It usually takes two steps to achieve this goal: a) identify which PK-PD index ($fAUC/MIC$, fC_{max}/MIC , $fT>MIC$) best predicts *in vivo* antibacterial activity and b) determine the magnitude of the PK-PD index required for *in vivo* efficacy. The advantages of the *in vitro* PD model are as follows: it can simulate human PK profile more easily than in animal models; it can produce multiple samples for investigating bacterial growth/kill kinetics, hence, it is more informative for discriminating the best index of bactericidal pattern, and is also a cheaper and effective way to study bacterial resistance; it is particularly useful to study bacterial strains that are difficult to grow *in vivo*; it minimizes the use of animals; and it is also useful in studying combination therapy.

Animal infection model also has its limitations: serum clearance of most antibacterials is faster in animals than in man; serum protein binding is usually less in animals than in man; the higher doses required for studies in animal models may result in non-linear kinetics. Computerized intravenous infusion pump has been used to simulate the PK profile seen in humans to lift some of the limitations but the cost of such an experiment is expensive^[21].

These analyses have been shown to be predictive of therapeutic success and failure against resistant microorganisms in clinical trials. The ability to provide a PK-PD target for clinical dosing has been particularly valuable for predicting outcomes against pathogens not encountered frequently enough in clinical trials to enable accurate conclusions.

2.1.1 *In vitro* kinetic models A useful approach to assess antibacterial efficacy is to use *in vitro* PD models based on time-kill curves. Time-kill curves can follow bacterial killing and growth as a function of both time and antibiotic concentration. Antibacterial concentration can either be held constant or changed to simulate an *in vivo* concentration profile, either in plasma or at the infection site. The resulting kill curves can be subsequently analyzed with appropriate PK-PD models. Finally these PK-

PD models then aid to optimize dosage regimens based on a rational, scientific approach. Various types of *in vitro* models have been devised. Generally speaking, there are two main types of *in vitro* PD models using either a constant antibiotic concentrations, which study the effects of a constant concentration of drug against bacteria as a function of time or variable antibacterial concentrations, in which the antibiotic concentrations fluctuate by dilution or diffusion.

2.1.1.1 Models with constant antibiotic concentration

These models study the number of bacteria exposed to a constant antibiotic concentration. Fig 2 shows *in vitro* time-kill curves, ranging from 0.25 to 4 times the MIC, that show the bactericidal pattern of activity of gentamicin and ampicillin against susceptible Gram-negative bacilli^[22]. Gentamicin displays a concentration-dependent pattern of bactericidal activity, whereas ampicillin displays a time-dependent pattern. Note that, when gentamicin concentrations increase, so too does the rate (slope steepness) and extent (change in log₁₀ CFU/mL) of bacterial killing across a wide range of exposure. In contrast, increasing ampicillin concentration results in an increased kill rate over a relatively narrow range of exposure. For ampicillin, there is no real increase in killing with higher concentrations of antibiotic once a concentration of twice the MIC is achieved.

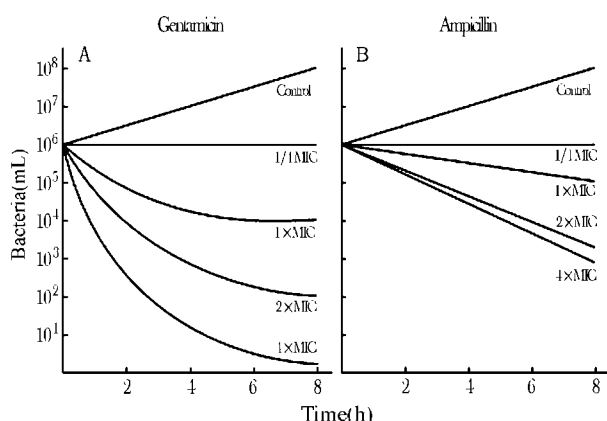


Fig 2 Time kill curves for the *in vitro* assessment of the effects of two different classes of antibiotics against susceptible Gram-negative bacilli: gentamicin (A) demonstrates concentration-dependent killing, whereas ampicillin (B) demonstrates time-dependent killing.

Curves in the presence (kill curves) and absence (growth curves) of antibacterial were compared. After an initial lag time, bacteria typically show a phase of logarithmic growth (log phase) that can be described by:

$$N = N_0 e^{-k_0 t} \quad (1)$$

where N is the number of bacteria at any given time point, N_0 is the number of bacteria in the initial inoculum, and k_0 is the first-order growth rate constant. N and N_0 are usually expressed in CFU per milliliter. Exposure to an antibiotic while the bacteria are in log phase will produce a change in k_0 , resulting in a different growth rate constant. A more generalized approach for concentration based PK-PD analysis is based on an E_{max} model. The rate of change in bacteria versus time (dN/dt) can be described by the following expression:

$$\frac{dN}{dt} = \left(k_0 - \frac{k_{max} \cdot C}{EC_{50} + C} \right) \cdot N \quad (2)$$

where N is the number of bacteria in CFU/mL, k_0 is the bacterial growth rate constant in the absence of drug, k_{max} is the maximum bacterial kill rate, C is the drug concentration, and EC_{50} is the drug concentration necessary to achieve half of the maximum effect.

A disadvantage of these approaches is that they do not reflect the *in vivo* situation where drug concentrations fluctuate. In addition, the above equations cannot describe the bacterial regrowth overtime due to the fixed concentration. As shown in Fig 3, the bactericidal activity of levofloxacin was found to be concentration-dependent. With increasing concentrations of levofloxacin used, a faster killing rate and a greater extent of killing were seen. Regrowth is evident after the initial reduction in bacterial burden in almost all time-kill studies.

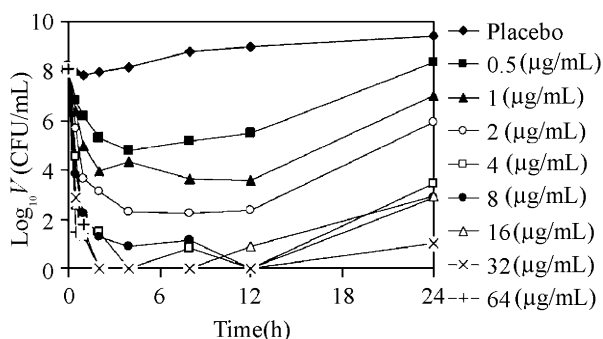


Fig 3 Time-kill studies of levofloxacin against *P. aeruginosa* TCC 27853 (MIC = 2 µg/mL). For $C = 32 \times \text{MIC} = 64 \mu\text{g/mL}$ there are no points beyond 1 h, since all bacteria appear to have been eradicated beyond that point in time^[20].

Therefore, the above mathematical descriptors of antibacterial behavior under constant concentration condition have limited ability of predicting or correlating with clinical outcomes. Nikolaou *et al* 2007 recently developed a

mathematical model to describe heterogeneous microbial population exposed to a time-invariant agent concentration (Fig 4). Based on the model and its parameter estimates, a three-dimensional response surface can be generated (Fig 5). It is evident that for a dosing interval of 24 h, a 2126 mg daily dose is required for eradication of the most resistant bacterial subpopulation, hence of the entire population as well. It is also evident that the daily dose of 750 mg recommended in standard literature is going to be inadequate, according to the mathematical model. The mathematical model has been validated in a hollow-fiber *in vitro* experimental infection model (see below).

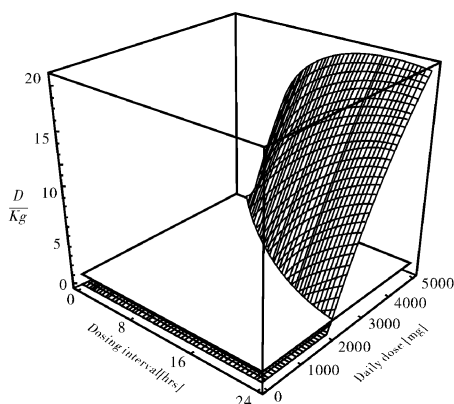


Fig 5 Mathematical model prediction of bactericidal effect of levofloxacin for bacterial population of *P. aeruginosa* using the PK-PD data collected from an *in vitro* time-invariant concentration experiment^[20].

2.1.1.2 Models with variable antibiotic concentrations

Models with changing antibiotic concentrations, on the other hand, try to simulate *in vivo* concentration-time profiles using human PK parameters in order to assess the an-

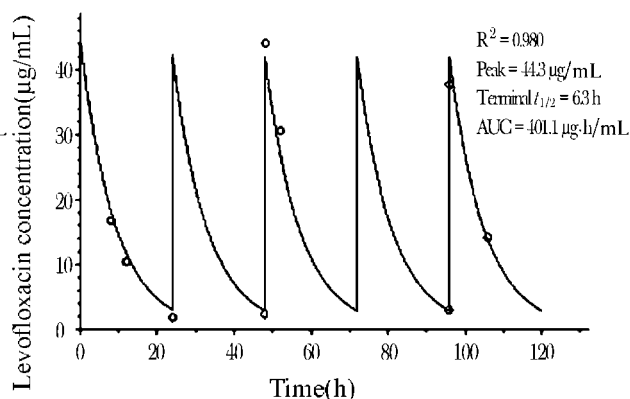
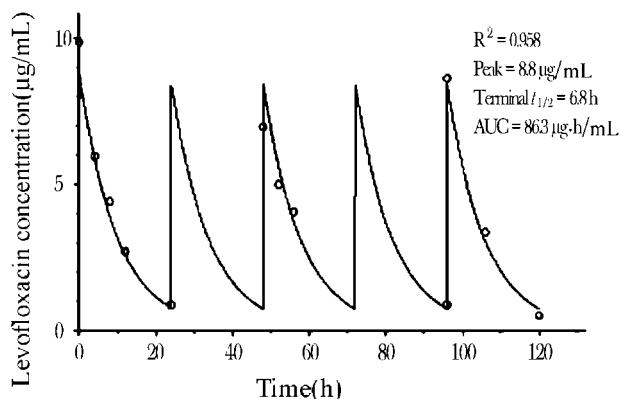


Fig 6 Simulated levofloxacin PK profiles: observed for daily dose of 750 mg (A), 3000 mg (B) given every 24 hours^[20].

Because the C in equation 2 now changes over time, both bacterial killing and regrowth can be described. The PD can be modeled from a pure PK perspective.

The PD data in the hollow-fiber model validated the mathematical model developed using the simpler static

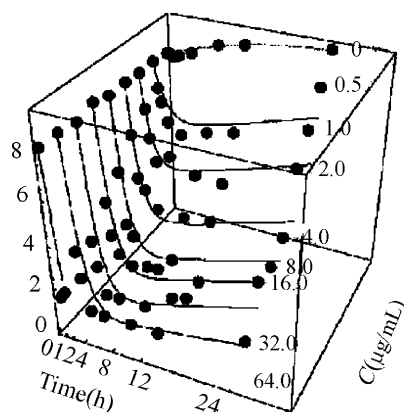


Fig 4 PD modeling of heterogeneous microbial population under time invariant antibacterial concentration^[20].

Dosing regimens(daily dose and dosing interval) associated with resistance suppression correspond to $D/K > 1$, where D represents time-averaged kill rate coefficient, K is the physiological microbial growth rate per microbial unit (net effect of natural microbial growth and death).

tibacterial effect. Changing concentrations can be produced either by dilution or diffusion. As shown in Fig 4, the human PK profiles of levofloxacin are simulated in the hollow fiber infection models.

concentration *in vitro* model. As shown in Fig 7, placebo control did not exert a selective pressure on the bacterial population. Therefore, no resistant subpopulation was detected over the duration of the experiment (Fig 7A). With the simulated clinical dose (750 mg given once dai-

ly), a significant killing of the bacterial population was observed at 4 and 8 h. However, regrowth was apparent with repeated dosing beyond 24 h, similar to that observed in time-kill studies. Regrowth observed over time was likely due to selective amplification of pre-existing resistant mutant resistant sub-population(s) likely to be present at baseline, as demonstrated in Fig 7B. This is consistent with the modeling approach. Susceptible bacterial populations were selectively eradicated, resulting in

unopposed growth of resistant sub-population(s) and consequently the enrichment of the total bacterial population by the resistant sub-population. As a proof of concept, a supra-clinical dose (3000 mg given once daily) above the threshold exposure for resistance development was simulated to verify if resistance in *P. aeruginosa* could be counter-selected. As predicted, sustained killing of the total bacterial burden and suppression of resistant sub-population was achieved over 5 days (Fig 7C).

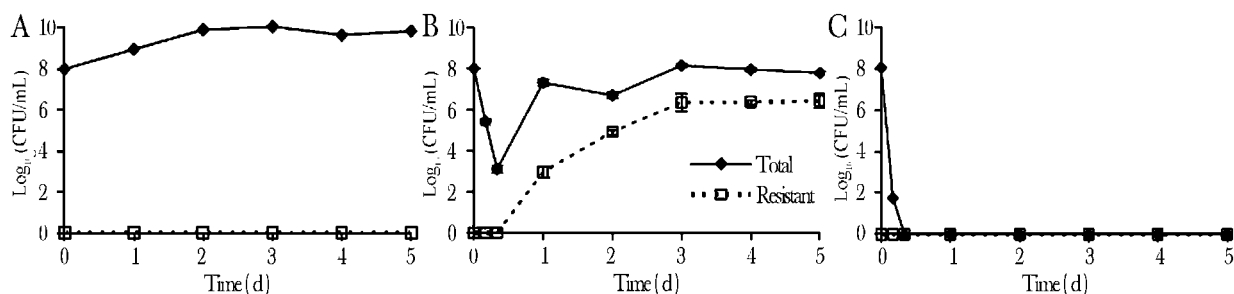


Fig 7 Prospective validation of the mathematical model in the hollow-fiber infection model for placebo (A), levofloxacin 750 mg (B), levofloxacin 3000 mg (C) given every 24 h. Data are presented as mean and standard deviation of duplicate samples.

Another important advantage of *in vitro* PD models is that it is possible to mimic effect site PK. Several *in vitro* models were developed that simulate *in vivo* conditions in specific infection sites or conditions, such as an bladder, bacterial cystitis, otitis medium, endocarditis, chronic pneumonia, tuberculosis, infected fibrin clots, and implant-related infections^[18]. However, such attempts to more closely mimic an *in vivo* situation and to predict clinical response were hampered by the inability to measure PK at the site of infection. Measuring target site PK has recently become possible by microdialysis, a technique which allows for the on-line measurement of unbound drug concentrations in the interstitial space. Since microdialysis monitors free antibacterial concentrations in the fluid which directly surrounds the infective agents, the antimicrobial effect linked to the time versus drug concentration profile obtained by microdialysis may easily be simulated in an *in vitro* setting on bacterial cultures. This dynamic simulation may provide a rational approach to describing and predicting PD at the relevant target site.

2.1.2 *In vivo* infection models The major goal of the PK-PD studies using animal *in vivo* infection models is to establish the PK-PD target required for effective antibacterial therapy-this usually takes two steps: a) identifying which PK-PD index best predicts *in vivo* antibacterial activity, b) determining the magnitude of the PK-PD index required for *in vivo* efficacy.

2.1.2.1 Discrimination of PK-PD indices best correlating with efficacy Animal model studies have a distinct advantage over clinical trials in their ability to discern which PK/PD dosing index is most closely associated with efficacy. In clinical trials, usually only 1 dose and 1 dosing interval are studied, making discrimination of the predictive PK-PD index impossible. This is because a colinearity exists between measures-that is, when the dose increases, so too does $T > MIC$, C_{max}/MIC , and AUC/MIC . One way to break the colinearity and identify the PK-PD index most closely associated with efficacy is through the use of dose-fractionation studies in preclinical setting. In such studies, the same total drug exposure is administered using different dosing intervals. For instance, a dose might be delivered as 1 g once daily or in 4 equally divided doses throughout the day. Regardless of dosing interval, each regimen would have identical AUC_{24}/MIC values, but different $T > MIC$ and C_{max}/MIC values. Most times, in order to break the colinearity, dose escalation in conjunction with dose fractionation needs to be applied. Fig 8 shows the results of a dose-fractionation study that evaluated the PK-PD profile of gatifloxacin against *Salmonella enterica* serotype Typhi in an *in vitro* PK-PD infection model^[2]. Clearly, the $fAUC/MIC$ is the best predictor of the efficacy. The correlation between efficacy and each of the three PK-PD indices studied (total and free-drug $T > MIC$, AUC/MIC , and

peak MIC) is usually determined by nonlinear least-squares multivariate regression. The coefficient of determination, or R^2 , was used to estimate the variance that

could be due to regression with each of the PK-PD indices.

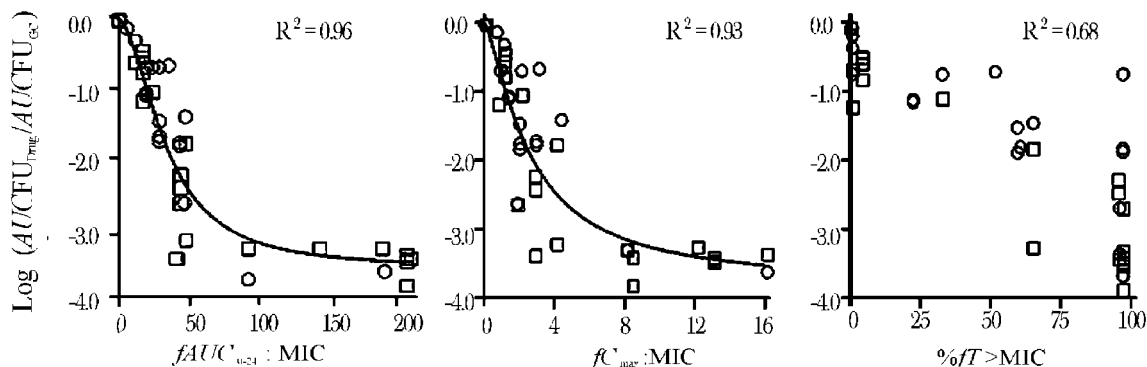


Fig 8 Relationships for gatifloxacin between the $fAUC/MIC$ (left), fC_{max}/MIC (middle), and $\%T > MIC$ (right) for 2 strains of *Salmonella enterica* serotype Typhi with differing MIC values and changes in bacterial density.

On the other hand, as shown in Fig 9, the *in vivo* activity of ceftazidime against *K. pneumoniae* in a murine

thigh infection model correlated best with the $f\%T > MIC^{[14]}$.

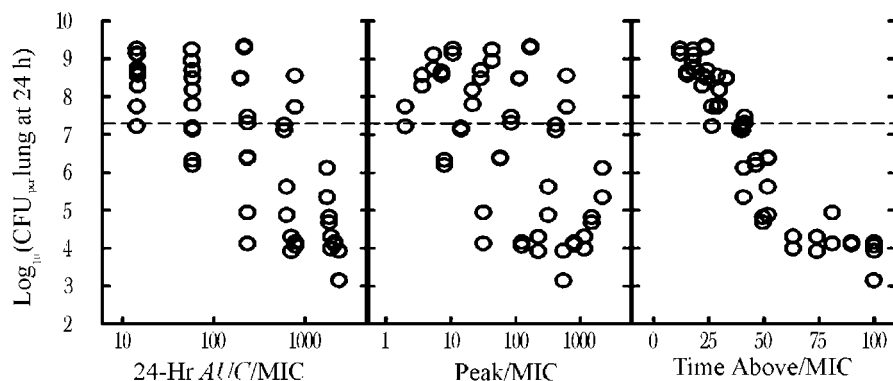


Fig 9 Relationship among PK/PD indices for ceftazidime and \log_{10} CFU per lung of *K. pneumoniae* after 24 h of therapy.

2.1.2.2 Magnitudes of the PK-PD index required for efficacy The PK-PD data of the animal infection model studies are usually analyzed using the sigmoid dose-effect model (Fig 10). The model is derived from the Hill equation:

$$E = \frac{E_{max} \cdot D^N}{ED_{50} + D^N} \quad (3)$$

where E is the effect or in this case the log change in CFU per thigh or lung, comparing treated mice and untreated controls after the 24-hour period of study. E_{max} is the maximum effect. D is the 24-hour total dose. ED_{50} is the dose required to achieve 50% of the E_{max} , and N is the slope of the dose-effect curve. The indices E_{max} , ED_{50} , and N are calculated using nonlinear least-squares regression.

The magnitude of the PK-PD index associated with each endpoint dose can be calculated from the following equation:

$$\log_{10} D = \frac{\log_{10} [E / (E_{max} - E)] + \log_{10} ED_{50}}{N} \quad (4)$$

where E is control growth (D equals the dose), $E = \text{control growth plus 1 log when } D = 1 \text{ log kill}$, or $E = \text{control plus 2 log for } D = 2 \text{ log kill}$.

Animal models are particularly useful for determining PK-PD magnitude necessary for treatment efficacy (target), subsequently, determining the dose regimen for clinical trials via Monte Carlo simulations. Clinical trials themselves are most often not able to define the optimal dose level, and it is very costly if do it in a trial-and-error manner. Animal studies are able ethically to define the entire dose-response relationship and thus better define the PK-PD target predictive of treatment outcome. Furthermore, a variety of studies have suggested that the magnitude of the PK-PD index associated with efficacy is similar among various animal species including humans^[23]. This demonstrates the ability of PD to correct for

kinetic variability among species. Serving as a surrogate, the determination of effective PK-PD target in various animal models has been especially useful for design of optimal dosing regimens in scenarios that are encountered infrequently in clinical trials^[23,24]. For example, most clinical trials are unable to enroll enough patients with resistant pathogens. However, animal model studies are able to define the relationship between index magnitude and effect against organisms with widely varying MICs^[24]. In addition, these studies can define efficacy against organisms with reduced susceptibility due to specific resistance mechanisms.

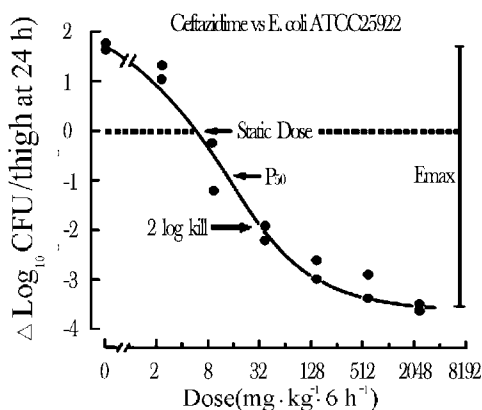


Fig 10 Mathematical analysis of dose response data from animal models after 24 hours of therapy

2.1.2.3 Suppression of resistance as an endpoint Bacterial populations in nature are heterogeneous with respect to their susceptibility to antibacterials. The presence of this smaller, more resistant bacterial subpopulations has important implications for chemotherapy (Fig 11). Current preclinical methods for assessing the efficacy of an antibacterial agent most often evaluate the effect of selected dosages of the compound on the reduction in the total bacterial population at an infection site. The impact of drug pressure on the amplification of a drug-resistant subpopulation is ignored^[25]. Recently, suppression of the emergence of resistance has been used as the endpoint in a mouse infection model study using *P. aeruginosa* and treated with a fluoroquinolone (Fig 12). A mathematical model was developed that described relationships between antibacterial drug exposures and changes in drug-susceptible and -resistant bacterial subpopulations at an infection site^[25]. Based on the model, an exposure that would optimally amplify the resistant subpopulation (AUC/MIC ratio=52 :1) and an exposure (157 :1) that was predicted to hold the population at or near the numbers present at

therapy initiation were derived. The mathematic model was prospectively validated in another experiment with a time frame longer than that examined in the original one (48 vs. 24 hours). Fig 13 shows the predicted time course of the total and resistant bacterial subpopulations (lines) and also the observed values from the validation experiment superimposed on the predicted lines. The likelihood of achieving resistance-suppression exposure in humans with a clinically prescribed antibiotic dose was determined. The methods developed in this study provide insight regarding how mathematical models can be used to identify rational dosing regimens that suppress the amplification of the resistant mutant population.

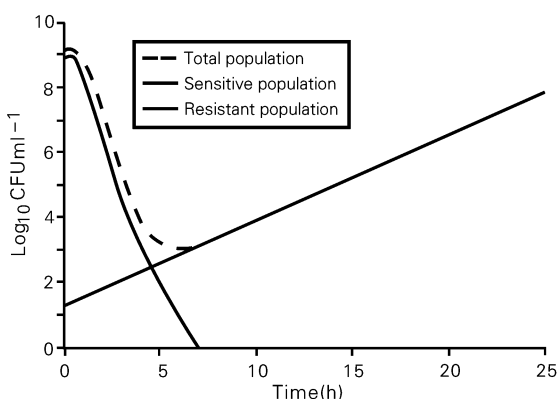


Fig 11 The differential effects of drug exposure on the two populations: the drug sensitive population is efficiently killed, whereas the drug resistant population is often amplified. The change in profile of the total population is explained by the differential effects of the drug exposure on the two populations^[27].

2.1.2.4 Factors affecting PK-PD target Animal model studies have also been useful for determining the factors that potentially influence PK-PD targets. These variables include drug classes, pathogen species, drug-resistant pathogens, site of infection, the treatment endpoint, and various host immune defects. Multitudes of studies have demonstrated that the PK-PD target predictive of outcomes is similar for drugs within the same drug class and for most pathogens^[23]. Different types of β -lactams, such as penicillins, cephalosporins and carbapenems, require free drug to be present for differing fractions of the dosing interval to achieve a Bacteriostatic effect or maximal bactericidal effect. For bacteriostasis, the concentration of free drug must exceed the MIC for 35%–40%, 30% and 20% of the dosing interval for cephalosporins, penicillins and carbapenems, respectively. Achievement of the maximal bactericidal effect requires 60%–70%, 50% and

40% coverage, respectively, for these β -lactam classes^[23]. Fig 14 illustrates the AUC_{24}/MIC with total and free drug for the static dose of different fluoroquinolones

with *S. pneumoniae* ATCC 10813. For the fluoroquinolones, the PK-PD targets (AUC_{24}/MIC) are quite similar (~ 30) once they are converted to unbound fraction^[23].

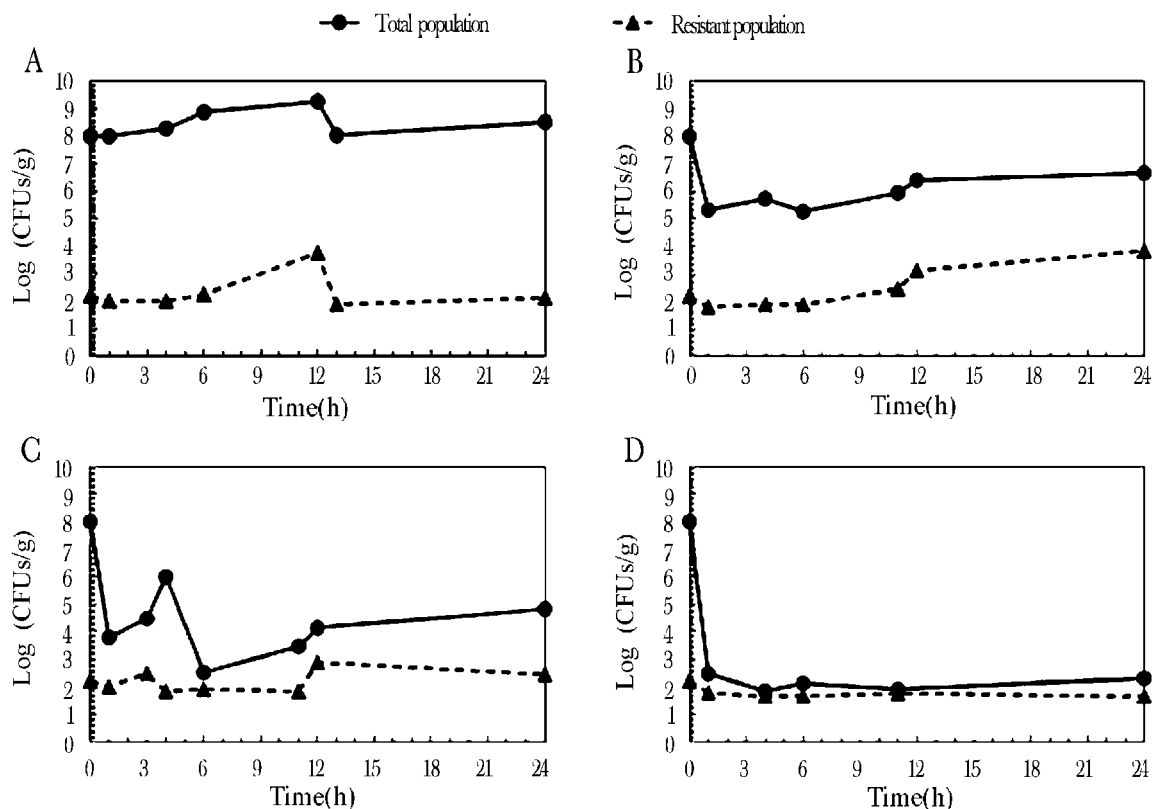


Fig 12 The effects of incremental levofloxacin exposures on the drug-susceptible and -resistant bacterial populations for four drug dosages(0, 90, 215, and 600 mg/kg, A—D, respectively). In the placebo group (A), the proportion of drug-susceptible to drug-resistant populations remained stable throughout the 24-hour study. A dose of 90 mg/kg of levofloxacin reduced the overall bacterial density. However, this dose amplified the resistant subpopulation(B). Higher doses of drug reduced the total bacterial population while holding the resistant mutants at their base-line concentration(C and D)^[25, 27].

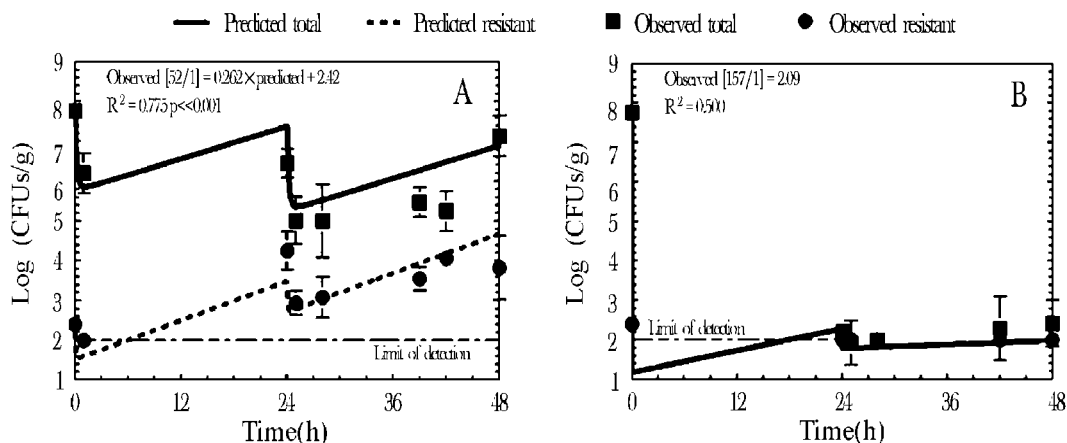


Fig 13 Levofloxacin prospective validation. The model predicted exposure that would encourage selection of resistance (A) or suppress emergence of resistance (B) were examined in an experiment. The experimental data confirmed the predictions^[25, 27].

The actual endpoint used for measuring antimicrobial activity can also influence interpretation of results. Many studies in the past have used survival as the major end-

point for treatment outcome. However, organism counts, especially those performed after 24 h, will often provide a wide range of responses that allows for use of lower num-

bers of animals to differentiate between treatment outcomes. Several studies have demonstrated a strong relationship between bacterial numbers and survival. As shown in the left panel of Fig 15, the change in log₁₀ CFU per lung after only 24 h of twice daily therapy with gentamicin against *K. pneumoniae* was very similar to the survival data obtained after 5 days of twice-daily therapy^[14]. The right panel of Fig 15 shows that there is an excellent correlation between the static dose derived from bacterial numbers at 24 h and the daily dose resulting in 50% survival on day 5. The symbols represent data with various β -lactams, fluoroquinolones and aminoglycosides against *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

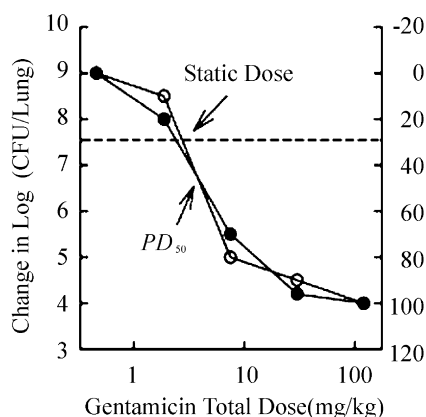


Fig 15 Relationships between change in CFU per lung at 24 h and survival at day 5 with twice daily therapy with gentamicin (left panel) and between the 24 h static dose and the dose protecting 50% of mice from death (PD_{50}) for various β -lactams, fluoroquinolones and aminoglycosides against *E. coli*, *K. pneumoniae* and *P. aeruginosa* (right panel).

The impact of neutrophils has been documented by many studies. The magnitude of the static dose was reduced 3–4 fold for macrolides and clindamycin and 5–6 fold for fluoroquinolones against *S. pneumoniae* in non-neutropenic compared with neutropenic mice^[13]. For Gram-negative Bacilli, the effect of neutrophils is minimal with beta-lactams, and is mild with fluoroquinones and aminoglycosides (less than 2 fold).

3 CLINICAL DEVELOPMENT

One of the most challenging issues in the design of phase II/III clinical trials of antibacterial agents is dose selection. Phase I PK studies are traditionally followed by phase II dose-finding studies wherein the various dosing regimens are arrived at empirically. However, in antibacterial clinical development, the traditional approach is not only time consuming but also often leads to no conclusion. Recently, there has been much progress in understanding the relationship between the dose and therapeutic efficacy

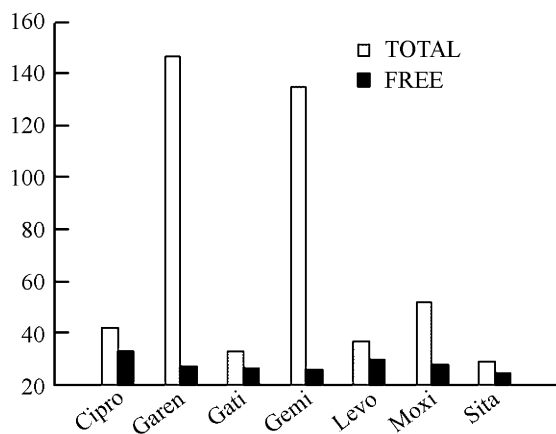
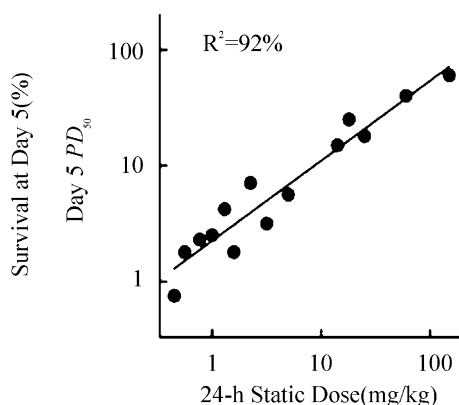


Fig 14 AUC_{24}/MIC with total and free drug for the static dose of different fluoroquinolones with *S. pneumoniae* ATCC 10813.



of antibacterial agents. Therapeutic efficacy has been shown to be dependent on particular PK-PD indices, i. e., either AUC/MIC and/or C_{max}/MIC or $T > MIC$ ^[23]. The relationship between PK-PD index and therapeutic efficacy has been shown for various antibacterial agents using *in vitro* PK and animal models. Drusano first presented a method which bridges preclinical data to Phase II/III using of Monte Carlo simulation for drug dose choice and breakpoint determination in 1998^[24]. The rational dose-selection paradigm which Drusano laid out was to integrate a) the distribution of MICs for clinical isolates, b) the distribution of the values of the PK parameters for the test drug in the population, c) the PD target(s) developed from animal models of infection, and d) the protein binding characteristics of the test drug.

According to this dosage selection strategy, it is first necessary to decide what magnitude of effect (PK-PD target) is desired from the drug dose chosen. This choice is usually based on clinical circumstances. For instance,

treatment of a minor community-acquired infection might only require that the drug achieve a static effect, so that the patient's intact immune system can clear the infection. However, treatment of a severely granulocytopenic patient with sepsis will require a near maximal bactericidal effect (e.g., 2 log kill). The relationship between measures of drug exposure and the microbiological effect that is achieved, as determined in a neutropenic mouse thigh infection model, is shown in Fig 10^[27].

Once a PK-PD target is selected, various questions can be posed: a) what is the highest MIC that will reliably achieve the PK-PD target for a specific drug dose in a population of patients? This is a method by which a breakpoint MIC value can be determined for the effect desired; b) how useful will a specific drug dose be, both in the target patient population and over the full range of MIC values from the clinical isolates?

The Monte Carlo simulation allows delineation of the

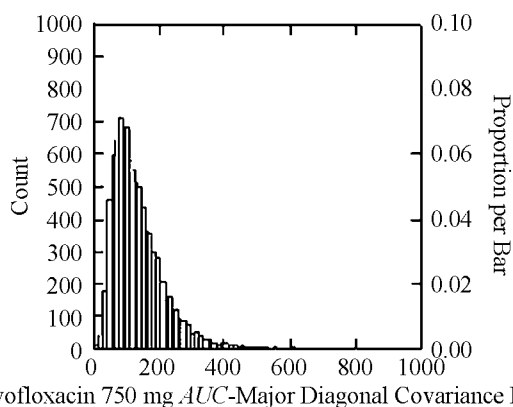
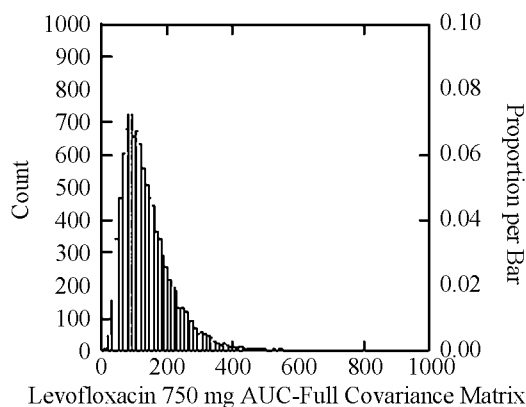


Fig 16 Monte Carlo simulation of PK parameter distribution using full covariance matrix and major diagonal matrix

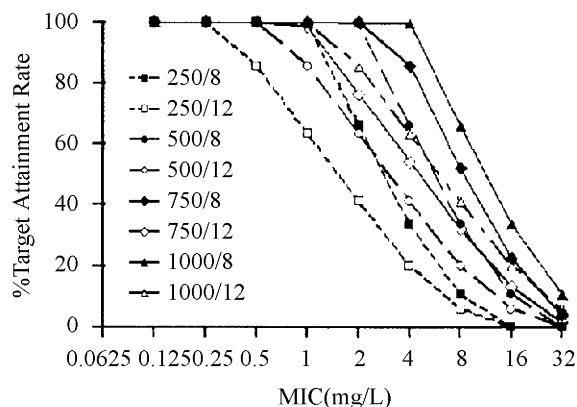


Fig 17 Probability of target attainment rate for various dosing regimens of a drug

Sparse sampling coupled with population PK modeling has been implemented in to clinical drug development to first establish a population PK model and then to esti-

mation of PK parameters (e.g., C_{max} and AUC) that would be seen in a large population after the use of a specific drug dose. Full covariance matrix or the correlation among parameters should be considered when conducting the simulation. Missing the off-diagonal terms often can cause the distribution to be broader with troublesome outlier values (Fig 16). These total drug values should be corrected for any protein-binding differences between the animal or *in vitro* model in which they are developed and those seen in humans. For each MIC value in the distribution of a large collection of target pathogens, the target attainment rate can be determined in the population of simulated subjects. This provides an answer to Question A (Fig 17). Because the fraction of the organism collection at each MIC value is known, a weighted average (expectation) of the target attainment rates can be taken. This value provides an answer to Question B.

mate the drug exposures for individual patients using maximal A-posteriori probability (MAP) Bayesian estimation. Both clinical and microbiological outcomes are usually determined for the patients. As these are dichotomous outcomes, logistic regression analysis is often used to link measures of drug exposure to the probability of a good clinical or microbiological outcome. Classification and regression tree (CART) analysis is an exploratory data-analysis technique that is a powerful way to examine how different factors interact and can influence outcome^[28]. However, in the area of anti-infective PD, this tool is particularly useful for determining exposure breakpoint values. For endpoints that are continuous variables, such as the number of organisms at a primary infection site, measures of exposure can be linked to the effect through a sigmoid-Emax effect model^[27].

4 OVERALL SUMMARY

Antimicrobial PD-the field that integrates microbiology and pharmacology-is an area that has seen a huge growth in knowledge over the past decades. The unique feature of this area is that we are able to isolate the pathogen and determine a measure of potency of the drug in question for this pathogen. Once measures of exposure (PK-PD target) can be linked to effect (it is often done in animal model or *in vitro* model), it is possible to choose drug doses that have a high likelihood of achieving the desired goals of therapy clinically. These relationships are now being elucidated more frequently owing to the broad availability of sophisticated PK-PD software and the application of appropriate statistical techniques to the data sets. The true aims of anti-infective therapy are to administer a dose of drug to a patient that will have an acceptably high probability of attaining the desired therapeutic effect, while also having an acceptably low probability of concentration-related toxicity. Owing to our ability to develop such relationships, we can now approach this long-desired goal of therapy and improve the outcomes of infection for our patients. As the development of concentration-effect and concentration-toxicity relationships becomes more common, the main regulatory agencies responsible for overseeing drug development should incorporate this process into the regulatory development requirements. In this way, the best balance between risk and benefit for the ill patient requiring drug administration can be attained.

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药物动力学和药效动力学结合 在抗菌药物研发和临床治疗中的应用

摘要 面临耐药性致病菌的逐年增加, 新型抗菌药物亟待开发。众所周知, 新药开发是一个艰难和复杂的过程。制定合理给药方案是药物开发的重要课题, 而以制定抗菌药物的合理给药方案最富挑战性。近二十年来, 便宜快捷的体外动力学实验和动物体内感染模型以及药效学数据处理方法不断完善, 其价值在抗菌新药开发上被不断验证和肯定, 弥补了临床试验所无法获得的信息。近十年来临床群体药代动力学模型和蒙地卡罗模拟结合临床前实验确定

的靶值, 致病菌 MIC 分布, 利用电脑模拟比较不同给药方案的中靶率, 已成为临床三期试验确定最佳给药方案行之有效的方法和针对耐药菌抗菌药物临床剂量再评价和进一步调整的科学依据。可以预期, 在今后的抗菌素新药的开发上, 人们将更有把握从临床前的实验数据来预计临床最佳给药方案, 并优化临床试验的设计, 达到既快速又经济的目的。

关键词 药动学; 药效学; 抗菌素; 体外动力学模型; 动物模型; 蒙地卡罗模拟