PK/PD Approaches

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Abstract

The success of the drug development program heavily relies on the rational drug design with appropriate choice of drug and dosing regimen. This requires a good understanding of both drug delivery mechanism and drug response mechanism. Two of the most important pharmacologic disciplines, namely, pharmacokinetics (PK) and pharmacodynamics (PD), can be linked together by PK/PD approach, which has tremendous potential to influence decision-making through modeling and simulation. With its nature of an interdisciplinary science, this state-of-art strategy can leverage different

Willi Weber has retired.

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kinds of preclinical and clinical data through mathematical and statistical models. This framework is powerful to assist researchers with better understanding of drug behavior and effectiveness, disease progression, and the impact of demographic characteristics on a subpopulation or individual patients. The aim of this chapter is to provide an overview of basic concepts in PK and PD and discuss various approaches in PK/PD modeling and simulation, together with its applications in antibiotic drug development as it is very well established in this field. The PK/PD concepts and theories presented here are not limited to antibiotics only but can also be broadly applied to drug development in other therapeutic areas.

Purpose and Rationale

Pharmacokinetics (PK) describes the time course of the drug concentration in the body, normally in blood or plasma. Pharmacodynamics (PD) describes the relationship between drug concentration and response, desired or not desired. PK/PD is the link between PK and PD describing the time course of drug effect.

Reviews on PK/PD are regularly published (Derendorf and Meibohm 1999; Derendorf et al. 2000; Csajka and Verotta 2006), and the more specialized literature is becoming more and more abundant. The book of Gabrielsson and Weiner (2016) is an excellent introduction to PK/PD and modeling. This chapter is a comprehensive summary of the state-of-the art with references to basic and recent literature. PK/PD modeling is assessed by reviewing and discussing its application in antibiotic drug development.

Mathematical Models in Biology

Biology is complex, much too complex to be completely described in equations. And it is extremely difficult to obtain all the data needed for a detailed description. The target exposure or concentration may not be easy to be determined with the limited raw data from observation. Models are reductions of reality to a mathematical system that can be handled, for example, by a computer. Models are informatic descriptions of a true biological process. They are often the simplified representations but differ in the degree of simplification. Since the nature of simplification is based on the intended use of the model, therefore there is no right model but rather judged by "fitness for purpose" (Mould and Upton 2012). Model cannot be better than the data without using previous knowledge. A dataset will never be complete. Models will differ depending on the available data. And each model is part of the truth. The situation is not new. It is the morale of the famous parable of "the blind men and the elephant" probably originating from Asia a long time ago:

> Six blind men were asked to determine what an elephant looked like by feeling different parts of the elephant's body. The blind man who feels a leg says the elephant is like a pillar; the one who feels the tail says the elephant is like a rope; the one who feels the trunk says the elephant is like a tree branch; the one who feels the ear says the elephant is like a hand fan; the one who feels the belly says the elephant is like a wall; and the one who feels the tusk says the elephant is like a solid pipe. And they started arguing and fighting and could not find out what was the truth. Finally, a wise man explains to them: "All of you are right. The reason every one of you is telling it differently is because each one of you touched a different part of the elephant. So, actually the elephant has all the features you mentioned."

Depending on the data and the task, different parts of a system are described, and different conclusions can be drawn. The question about the use of a model under development decides on the data needed. What is the resolution in time, in space, and in concentration required? Will the model be used for interpolation only, or is it to simulate new situations by extrapolation? Pure empiric models can be used to interpolate in the limits of data used to develop the model. More mechanistic models are more difficult, but more useful when cautious extrapolation beyond the limits of observed data is required. Finally, models may change if additional data are available.

Modeling in Pharmacometrics

The modeler can use two principal types of models. One possibility is to fit a simpler empirical model with large sample size or exposure ranges. Such approach is useful for describing the underlining relationship. However, further extrapolation outside of the study population would not be straightforward if the parameters are not biologically interpretable. Hence empirical models are usually descriptive models which would provide adequate information to understand the response difference in the data. The other type of the model incorporates the essential pharmacological and physiological information into the model but still keeps it as simple as possible, which is also classified as predictive models. The modeling approach can start with a complex model where the complete physiological knowledge is expressed in mathematical equations and then simplify this complicated model to the level that it can be processed in a computer and the parameters are identifiable. This type of models can be intended to extrapolate the same relationship to other populations from whom data has not been used to derive the model. Stronger assumptions are usually made for this type of model. Therefore, model validation is particularly important to ensure the confidence of model application for its intended use.

To assess the credibility of modeling and simulation, both the fidelity and robustness of the model need to be considered. The fidelity of the model can be gauged by comparing the model structure and parameters to the real and important biological basis. And the robustness of the model can be addressed by parameter sensitivity analysis, variance estimation, and bootstrap resampling. With the evolving computational sciences and unprecedented availability of data in pharmaceutical sciences, building credible models to quantitatively assess and evaluate drug therapies becomes feasible. Model-based drug development, mainly initiated by population PK/PD, provides a more rational and efficient approach for dose selection and optimization.

One of the biggest challenges for data interpretation is the variability in exposure and response between individual subjects. The variability comes from the individual's physiologic characteristics such as age, weight, gender, etc. Understanding the impact of these covariates on PK parameters is particularly important in model development. The data collected at different time points for the same individual are correlated with each other; ignoring this correlation would lead to inflated variability in PK/PD model and biased covariate-parameter relationship. In order to ensure the robustness of parameter estimation, mixed-effect modeling is often used to appropriately controlling variability in model fitting. Mixed-effect modeling does not only calculate the PK/PD parameters but also their statistical distribution in the population and is therefore called population PK/PD in this context.

Procedure

Basic Concepts of Pharmacokinetics

Pharmacokinetics (PK) is also referred as "what the body does to the drug" including the processes of absorption, distribution, metabolism, and excretion that govern the concentration-time course of a drug in blood or plasma. In principle, non-compartmental, compartmental, and physiologically based models can be used for PK modeling. Most PK models use compartments as the building block to describe the concentration-time course, in most cases in the central compartment. The "compartment," a very common abstract concept in PK models, is assumed as a region of the body where the drug is homogenously mixed and shares similar kinetics. The central compartment usually represents plasma or the systemic circulation. However, the central compartment is not necessarily the site of action since most drug-target action occurs in tissues. The drug concentration at the site of action may be accessible to measurements, but very often it is not and has to be calculated in the PK models. For that reason, the PK models generally consist of a central compartment and one or two peripheral compartments linked by distribution rate constants.

Drug distribution into tissue depends on both plasma protein and tissue binding. In plasma, drugs can bind to proteins such as albumin, α-1 acid glycoproteins, and lipoproteins. Only the free, unbound drug can distribute into the tissue via passive diffusion and subsequently reaches its site of action in the tissue for pharmacologic effects. Therefore, the frequently measured total plasma concentration may not be an ideal measure, but the free concentration at the target site, if possible, should be used to derive PK models. Achieving and maintaining goal tissue concentration near the target site is critical to maximize drug effectiveness and minimize drug toxicity. One of the most frequently used methods to sample unbound drug concentration in the interstitial fluid (ISF) of various tissues is microdialysis (Chaurasia et al. 2007). Rather than obtaining average total tissue concentration after homogenization of tissue samples, microdialysis technique provides a less invasive and more direct way to continuously measure the unbound concentration in the tissue. In brief, a microdialysis probe is implanted into the tissue and continuously perfused with a perfusate. By measuring the dialysate sample and correlating the concentration in dialysate and tissue, the free drug concentration in the ISF can thus be calculated.

To clearly explain how clinical microdialysis works, an example from Schuck et al. is illustrated here (Schuck et al. 2005). Microdialysis technique was used to study the effect of simulated microgravity on the tissue distribution of ciprofloxacin. Six healthy volunteers participated in a crossover study after a single 250-mg oral ciprofloxacin in normal gravity and simulated microgravity. Blood, urine, and *in vivo* microdialysis samples were obtained from thigh muscle in each subject. After probe implantation, probe calibration by retrodialysis was performed before microdialysis started. Probe was perfused with 0.1 mg/L ciprofloxacin solution, and drug concentration in dialysate was measured. *In vivo* recovery of ciprofloxacin was determined by computing the disappearance rate through the semipermeable membrane at the tip of microdialysis probe using the following equation:

$$\text{Recovery}\ (\%) = \left(1 - \frac{\text{Concentration}_{\text{dialysate}}}{\text{Concentration}_{\text{perfusate}}}\right) \times 100 \quad (1)$$

After the completion of probe calibration and washout period, ciprofloxacin was then administered, and microdialysis sample collection started and continues with the predetermined 30-min time interval for each collection. Under the assumption of quantitatively equal diffusion process in both directions through semipermeable membrane, the same *in vivo* recovery was used to calculate free concentration in the tissue:

$$\text{Concentration}_{\text{tissue}} = \frac{\text{Concentration}_{\text{dialysate}}}{\text{Recovery}(\%)} \times 100 \quad (2)$$

The mean PK profile of total plasma and free tissue concentrations is presented in Fig. 1. It shows very similar total ciprofloxacin concentration in plasma under normal gravity and microgravity; however, slightly lower but not significantly different tissue concentration was observed in simulated microgravity. If total plasma concentration was used to estimate the therapeutic outcome of ciprofloxacin, the drug efficacy may be overestimated. With the successful measurement of free concentration in tissue by clinical microdialysis, this study suggested a slightly impaired tissue penetration of ciprofloxacin in microgravity. The free concentrations at the target site are better predictors of therapeutic outcome since these are the direct link between PK and PD.

Basic Concepts of Pharmacodynamics

The drug at the target site interacts with the target and hence results in active pharmacological action. The term pharmacodynamics (PD) describes "what the drug does to the body." In general, a drug effect E is depending on time t, drug concentrations C in the past until present, and additional covariates co through a function F:

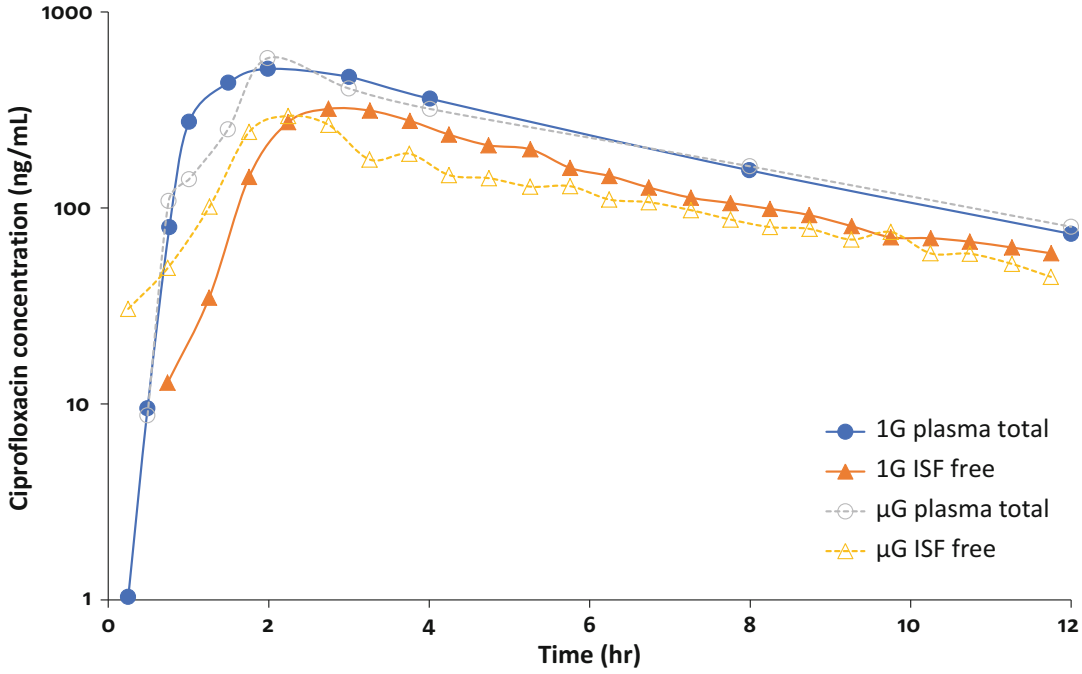

Fig. 1 Total plasma (circle) and free interstitial (triangle) concentrations of ciprofloxacin under Earth's gravity (solid) and simulated microgravity (open). Points represent the means of six subjects. (Image adapted from Schuck et al. (2005) and used with permission)

$$E(t) = F(t, C(-\infty,t), \mathrm{co}(-\infty,t)) \quad (3)$$

This is the description of a general dynamic system. In a static system, the effect depends only on the current time without any memory:

$$E(t) = F(t, C(t), \mathrm{co}(t)) \quad (4)$$

The system may be time variant or invariant. For an invariant system, the response to a drug concentration is always the same and does not depend on the time when it is applied. In a time-variant system, the response is depending on the time, for example, through time changing covariates co(t).

A relaxed system is at rest ($F = 0$) for drug concentrations $C = 0$:

$$F(t,0, \mathrm{co}(t)) \quad (5)$$

for all times $t \in [-\infty, \infty]$ and is excited ($F > 0$) by drug concentrations $C > 0$. For a linear system, the effect of the sum of two concentrations is the sum of the effect of each concentration. If the relationship between drug concentration and pharmacologic effect is linear, it can be expressed as a straight line:

$$E = S \bullet C + E_0 \quad (6)$$

with effect E, the slope of the line S, drug concentration C, and the effect in the absence of drug E_0. However, the relationship between concentration and effect may not be linear for the whole concentration range in most cases, especially at very low or very high drug concentrations. The linearly increase in drug effect also contradicts with the actual biologic response where a maximum response will be reached with ever-increasing drug concentration. Since many effects are nonlinear, so this simple additive procedure cannot be applied in most situations.

A well-known model that can simply and adequately describe the concentration-effect relationship is the E_{max} model:

$$E = \frac{E_{max} \bullet C}{EC_{50} + C} \tag{7}$$

where E_{max} is the maximum drug effect and EC_{50} is the concentration yields 50% of the maximum effect. This model can mimic the increasing trend of effect as drug concentration increased and is also able to predict the saturation effect at very high concentration levels. It is also important to notice that this model can be simplified to a linear relationship for small $C \ll EC_{50}$ such that $S = E_{max}/EC_{50}$ and to an inverse proportional relationship for large $C \gg EC_{50}$ such that $\left(E = E_{max} \times \left(1 - \frac{EC_{50}}{C}\right)\right)$.

In order to adjust the shape of effect-concentration relationship, a Hill factor is often incorporated into the E_{max} model that becomes the so-called sigmoid E_{max} model:

$$E = \frac{E_{max} \bullet C^{\gamma}}{EC_{50}{}^{\gamma} + C^{\gamma}} \tag{8}$$

where γ affects the slope of the middle part of the curve with flattened curve if $\gamma < 1$ and steeper curve if $\gamma > 1$. As γ increases, the effect becomes more sensitive to the change of drug concentration. When γ is very large (>5), the effect E turns out to be 0 if below the threshold EC_{50} and E_{max} if above EC_{50}. The concentration-effect relationship is almost like an on-off switch in such threshold model. Although this parameter rarely has mechanistic meanings, this value may help characterize and classify drug effect (Goutelle et al. 2008). For example, it has been shown that a high Hill coefficient and low maximum kill rate are observed for time-dependent antibiotics, while a low Hill coefficient and high maximum kill rate are seen among concentration-dependent antibiotics (Czock and Keller 2007).

Types of Pharmacokinetic Models

The most important component of model-based drug development is the pharmacokinetic model, which has different types: non-compartmental analysis, compartmental models, and physiological models. Non-compartmental analysis uses concentration-time data to estimate essential PK parameters such as AUC, CL, $t_{1/2}$, C_{max}, T_{max}, etc. with fewer assumptions than other model-based approaches. The accuracy of non-compartmental analysis increases with more time points in concentration-time data. Even though non-compartmental analysis has less predictability of PK profiles with different dosing regimen that has not been investigated, this approach has frequently been applied for allometric scaling and is also acceptable for bioequivalence studies.

The majority of PK models are compartmental models which rely on linear or nonlinear differential equations derived from mass balance to describe drug kinetics. Instead of assuming the body as one homogenous compartment, compartmental modeling divides the whole body into several interconnected compartments with each consisting of organs or tissues that are kinetically homogenous. It also assumes that the rate of drug distribution between compartments follows first-order kinetics. This approach is critically important since it can predict concentration-time profiles of alternative dosing regimen from simulation. Thus this simple modeling technique is quite useful in PK/PD approach to relate drug response mechanism with drug delivery mechanism. The most widely used modeling technique in PK/PD approach is population modeling, which will be discussed with more details later in this chapter.

The biggest limitation of conventional compartmental models is that different compartments may not have a clear physiological significance but are abstract mathematical constructs. To overcome this limitation and extrapolate PK to different physiological conditions or alternatively to drugs has similar property; physiologically based pharmacokinetic (PBPK) models were developed

based on actual physiological and biological meanings for drug and are expected to be a simple and direct approach to relate the observed drug response to target tissue exposure. Both organ physiology (weight, blood flow, enzyme expression, etc.) and drug-specific physical-chemical property (solubility, protein binding, tissue to plasma partition coefficient, etc.) are critical prior information for robust PBPK model development. A validated PBPK model can predict the quantitative behavior of similar drugs or extrapolate PK with altered physiology which is beyond the range of investigated experimental conditions.

Types of Pharmacodynamic Models

Limited conclusions can be drawn from PK alone; PK/PD approach bridges the gap between the time course of drug concentration and therapeutic response. PD response usually has several dependent variables as clinical endpoints, surrogates, and biomarkers. In most cases, only one is modeled at a time. It is an important decision to choose an appropriate dependent variable which is meaningful, measurable, and appropriate for modeling. Dependent variables may be continuous (blood pressure), categorical (several score levels), or binary (alive, dead).

Continuous Response Variables

The simplest PD model is a direct response model where the effect is directly related to the concentration in central compartment. Theoretically, the therapeutic response is directly triggered by drug concentration at effect site. The direct correlation between response and plasma concentration may result from rapid drug distribution and instant pharmacological response or the equilibrium between plasma and effect site achieved at steady state (Derendorf et al. 2000). This type of model can often be expressed using Eq. 8.

A delayed effect may be observed when there is a temporal dissociation between the time course of blood/plasma concentration and the effect of the pharmacological agent. This dissociation may occur due to the delayed distribution between central compartment and target site, or indirect mechanisms such as time-consuming synthesis or degradation of endogenous substance, or a more complex receptor-mediated effect (Derendorf et al. 2000). Indirect models are often used to describe this type of model where the relationship between response and concentration is not one-to-one but rather shown in a counterclockwise hysteresis loop.

In case of delayed effect, it is often that either an effect compartment model or an indirect response model can be used to describe it. An effect compartment is a hypothetical compartment characterized by the time course of concentration at the effect site. It is linked to the PK compartment but with no intercompartment mass transfer. The indirect response mechanism can be described using indirect response models where the drug affects a precursor and subsequently influences the PD response. The rate of change of the response over time in the absence of drug can be expressed as:

$$\frac{\mathrm{dR}}{\mathrm{dt}} = k_{\mathrm{in}} - k_{\mathrm{out}} R \tag{9}$$

where R is the measured response, k_{in} is the apparent zero-order rate constant for response production, and k_{out} is the first-order rate constant for response dissipation. Four basic models were proposed that the drug can either inhibit or stimulate the production or loss of the response (Sharma and Jusko 1996). Both the inhibition and stimulation functions can be incorporated into k_{in} or k_{out} in Eq. 9 with expression of $\left(I(C_p) = 1 - \frac{I_{\max} \bullet C_p}{\mathrm{IC}_{50} + C_p}\right)$ for inhibition and $\left(S(C_p) = 1 + \frac{E_{\max} \bullet C_p}{\mathrm{EC}_{50} + C_p}\right)$ for stimulation.

One of the possible reasons for the lag of drug effects may come from signal transduction controlled by secondary messengers (Mager et al. 2003). Other mechanistic PD models based on irreversible effects (e.g., cell or target inactivation, enzyme inactivation) and tolerance mechanism (e.g., counter-regulation, precursor pool depletion) are also important components and can play a big role in PK/PD approach.

Noncontinuous Response Variables

The majority of PD models describe continuous response variables. However, clinical data are not always continuous but sometimes categorical variables such as the severity of a disease (ordinal scaled), or binary variables (dead, alive), or time-to-event (censored) data. Such responses are more clinically relevant to drug efficacy and safety and can be described as the probability of an event occur using logistic models or survival models.

Logistic regression is suitable to use for the prediction of probability change with predictors when the outcome is a binary response. The logit transformation, the link function in logistic regression, is defined as the logarithm of the odds (the probability of an effect occurs divided by the probability of the effect not occur):

$$\text{logit}(p) = \ln\left(\frac{p}{1-p}\right) = L(x) \qquad (10)$$

where $L(x)$ is a linear function of predictors such as $L(x) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k$ where x_i represents the predictors (e.g., drug concentration, exposure, time). The logit transformation expands the ends of scale by mapping the interval (0, 1) onto $(-\infty, \infty)$, such that the small difference in p would reflect as a larger difference on logit scale. Instead of just p, logit(p) is used as the response in this regression. Its inverse function, also known as the logistic function, is as following:

$$p(x) = \frac{\exp(\text{logit})}{1+\exp(\text{logit})} = \frac{\exp(L(x))}{1+\exp(L(x))} \quad \text{or}$$
$$p(x) = \frac{1}{1+\exp(-\text{logit})} = \frac{1}{1+\exp(-L(x))} \qquad (11)$$

Both the logit and its inverse functions are monotonically increasing; the confidence interval for the probability of an event occurs (p) can be extrapolated from the confidence interval for logit (p). The application of logistic regression can also be extended in cases where the dependent variable has more than two outcome categories or when the multiple categories are ordered.

For the analysis of time-to-event data (such as time from origin of treatment to disease progression or duration of survival), they are often "censored" due to loss of follow-up (right censoring) or delayed entry (left censoring). Survival analysis aims to avoid this bias of incomplete longitudinal data. The survival function is defined as the probability that the event has not occurred by certain duration such that $S(t) = \Pr(T > t)$, where T denotes the time of the event occur. Also, notice that $F(t) = 1 - S(t)$ where $F(t)$ is the cumulative distribution function; this leads to the relationship of $-S'(t) = f(t)$, the probability density function of the lifetimes T. The hazard function $h(t)$ is the probability of an event occur immediately following time t given that the event has not occurred at time t:

$$h(t) = \lim_{\Delta t \to 0} \frac{\Pr(t < T < t + \Delta t | \, T > t)}{\Delta t} = \frac{f(t)}{S(t)} \qquad (12)$$

The cumulative hazard $H(t)$ is the area under the hazard function which relates to the survival function in the relationship as:

$$H(t) = -\ln S(t) \;\text{ or }\; S(t) = \exp[-H(t)] \qquad (13)$$

The survival function $S(t)$ is monotonically decreasing, while the cumulative hazard function $H(t)$ is monotonically increasing. When no event time is censored, the survival and hazard can be easily estimated by assuming parametric survival distributions (e.g., exponential, Weibull, log-logistic, etc.)

In case of data with right censoring or left truncation, nonparametric method should be used. Kaplan-Meier estimator and Nelson-Aalen estimator provide nonparametric estimates for survival function and cumulative hazard rate function, respectively. The Cox regression model, also known as the proportional hazards regression, is a semi-parametric model with an unspecified baseline hazard function (nonparametric) and a parametric component:

$$h(t|X) = h_0(t)\exp(\beta X) \quad (14)$$

where $X = (X_1, \ldots, X_n)$ is the covariate vector and $\beta = (\beta_1, \ldots, \beta_k)'$ is the coefficient vector with each β_i the log hazard ratio associated with one-unit change in X_i with the rest of covariates remaining constant. The baseline hazard only depends on t but not on any covariates X_i, while the hazard ratio $\exp(\beta X)$ only depends on the covariates but not on time t (independent of time, so-called proportional hazard). However, time-dependent covariates are more commonly seen in pharmacometrics setting since the hazard is associated with drug concentration which varies over time. In this case, the Cox regression model can still be used but the hazard ratio depends on time as well:

$$h(t|X) = h_0(t)\exp(\beta X(t)) \quad (15)$$

Gieschke et al. applied both logistic regression and Cox regression model to explore the relationship between systemic capecitabine exposure and its safety/efficacy outcomes (Gieschke et al. 2003). Holford discussed the details of the link between basic concepts of PK/PD and time-to-event analysis and how this approach can reveal more information for the prediction of therapeutic effects (Holford 2013). Gong et al. proposed the application of machine learning method for time-to-event data analysis, and their results indicated that machine learning-based methods provide better performance than the traditional Cox model (Gong et al. 2018).

Evaluation

Plotting effects versus various covariates like time, concentrations, and demographic variables will help to generate hypothesis about a future model. Is the effect time invariant? Is a hysteresis observed? It is Clockwise or counterclockwise? A strategy to decide about using an effect compartment or a direct response model has been discussed by Felmlee et al. (2012). Knowledge about the underlying process can also help to decide on this question and about the related physiology and will also help to explore more mechanistic models.

The following items should be clearly addressed in a PK/PD model development:

1. Problem and purpose of the model
2. Assumptions in the modeling process (explicit and implicit assumptions)
3. Rationale of model development (Why is a model considered to be better than another?)
4. Validation strategy using internal or external data

General Approaches of Pharmacometrics

All models are mathematical representations of the data. Often, the main objective of developing a PK/PD model is to describe the data, to predict unknown situations, and to explain underlying mechanisms. The application of using mathematics and statistics to understand data during drug development originates from simple descriptive summary of data. With the fast development in this field for the last 30 years, a lot of improvements have been seen in model efficiency and capability, especially after the inception of population modeling.

Traditional Approach

The simplest modeling approach to evaluate data from multiple subjects or animals is "naïve pooled approach," where data from all individuals are pooled first and then fit. The mean response estimated using this approach is always biased, and the interindividual differences in exposure and response are also ignored. Consequently, this is rarely used for PK/PD data analysis. Before the inception of population approach, the traditional method that modelers used is "two-stage approach." The individual's data are fit separately first. Subsequently, individual parameter estimates are combined, and descriptive summary statistics are calculated including mean, variance, and covariates on each parameter. The mean estimates of parameters obtained from this approach

are generally good, but the estimates of interindividual variability are biased and imprecise (Sheiner and Beal 1980). In addition, the two-stage approach highly relies on the richness of data for each individual. Hence this approach may not be applicable in the situation of sparse data where individual parameters are not easy to estimate. To overcome the limitation of both earlier approaches, Sheiner et al. developed a new approach which allowed dealing with sparse data to estimate population mean parameters and interindividual variability and incorporate covariate effects (Sheiner et al. 1972). With the advancement in computing power, this valid and robust population approach has been widely used in PK/PD modeling to facilitate current drug development.

Population Approach

The population approach, also known as the mixed-effect modeling, consists of both fixed effect parameters (population mean values) and random effect parameters (variability within the population). In general, all PK and PD parameters have certain distributions in population. Nonlinear mixed-effect modeling can simultaneously calculate parameters and their distributions from the full set of individual data. In this way, information from each individual are gathered and contributed to the covariate determination and the corresponding variability quantification. A general population model consists of three components: a structural model, a covariate model, and a stochastic model (Mould and Upton 2012). Structural model adopts the classical compartmental model to describe the time course of concentration profile (PK) or measured response (PD). Covariate model characterizes the relationship between PK/PD response to demographic covariates (such as weight, height, gender, etc.). The covariate identification and covariate model development are very critical as it supports labeling for special population based on their demographic information such as kidney/liver function, metabolic status, etc. Stochastic model describes the unexplainable variability in the data which includes inter- and intraindividual variability, residual, etc. More detailed discussion of population PK can be found in a separated chapter in this book. The application of population approach in PK/PD modeling provides clinical pharmacologist with better understanding of underlining mechanisms and assists rational dose adjustment for subpopulations of patients.

Learning and Confirming Circle

Drug development usually involves several iterations of model-informed learning and confirming where learning answers "how much/what" questions and confirming answers "yes/no" questions. Classical clinical studies are self-consistent, that is, a hypothesis is accepted or refused with the information coming from the study and nothing else. The learn and confirm approach (Sheiner 1997) consists of alternating learning and confirming cycles: a study to generate a hypothesis and a subsequent study to confirm the hypothesis (or to improve the model), followed by further hypothesis generating based on the previous finding and confirming (or not confirming) studies. Phase 1 and Phase 2B/3 are considered as learning stages, while Phase 2A and Phase 3/4 are seen as confirming stages based on the objectives in each stage. However, learning is still a very important subsidiary in the confirmation stages since learning while confirming would help keep the knowledge updated for confirming questions.

Software

Given that pharmacometrics is an interdisciplinary science fused with pharmacology, physiology, computer science, and mathematical/statistical modeling, PK/PD modeling and simulation heavily relies on software to make clinical trials more efficient. With the rapid evolving computation efficiency, a variety of efficient, flexible, and user-friendly software are available recently. For non-compartmental analysis, WinNonlin (Phoenix) has been widely used in pharmaceutical industry. Other software such as PK packages in R, Kinetica, Scientist, and PKSolver can also perform non-compartmental analysis. As for nonlinear mixed-effect modeling, NONMEM was the first and most commonly used software for

population PK and PK/PD modeling and simulation. Ever since the release of the first version, NONMEM has been continuously updated with new statistical methods and estimation algorithms. Because NONMEM does not have a very user-friendly interface, "front-end" and "back-end" software (such as Pirana, R, SAS) have been incorporated with NONMEM to overcome this limitation. Other software which has been widely used for mixed-effect modeling include MONOLIX, a software based on the stochastic approximation expectation maximization (SAEM) algorithm for reliable convergence and ADAPT, which is based on Monte Carlo parametric expectation maximization. Mixed-effect modeling is also possible in R with packages such as NLME, RxODE, mrgsolve, saemix, etc. The Pmetrics package in R allows nonparametric modeling of population PK using nonparametric adaptive grid (NPAG) algorithm. In addition, packages like PKgraph and ggplot provide graphical user interface for model diagnosis. The biggest advantages of R are the various statistical packages available and the possibilities of data management and visualization and that many scientists working in population PK/PD are programming with R and are publishing solutions for various problems. Berkeley Madonna also serves as a fast ordinary differential equation solver with a visualization interface. Besides NONMEM, Bayesian pharmacometrics modeling can also be performed in BUGS and Stan. SimCyp, PK-Sim, and GastroPlus are commonly used for PBPK modeling. Versions and owners of software change rapidly. Actual information about these commercial or free software packages can be found on the corresponding web sites on the Internet.

Critical Assessment of the Method

PK/PD Concepts in Antimicrobials

Establishing PK/PD relationship of drug candidates is critical during the whole process of drug development. Bridging the PK/PD information from preclinical to clinical studies and optimizing dosage regimen for special population is very crucial to ensure drug efficacy and to minimize toxicity. In this section, we will introduce important concepts of PK/PD approach in antimicrobial drug development since the PK/PD approach in this area has been well developed. The determination of correct dose and dosing interval can be evaluated via PK/PD approach (Sy et al. 2016). The discussion of PK/PD approach in antibiotic dose optimization is mainly separated into three approaches, namely, the minimum inhibitory concentration (MIC)-based, the time course-based, and *in vivo* animal model-based approaches. The PK/PD concepts and theories presented for antibiotics are also broadly applicable to drug development in other therapeutic areas.

MIC-Based Approaches

MIC, defined as the minimum inhibitory concentration, is a PD surrogate index of the susceptibility of certain pathogen in the presence of a specific antimicrobial treatment. This simple index does not reflect the time course of pharmacological effect but provides very useful information on antibiotic efficacy. From *in vitro* experiment, MIC can be easily determined as the lowest antibiotic concentration that inhibits the visible growth of microorganism in the end of a 16–20-h incubation period (Sy and Derendorf 2016). The most common MIC-based PD indices (Fig. 2) that quantitatively link the drug exposure and microbiological outcomes for *in vivo* efficacy prediction are the percentage of a dosing interval in which the drug concentration is above the MIC ($T > MIC$, time above MIC), the ratio between the peak concentration and MIC (C_{max}/MIC), and the 24-h area under the concentration-time curve divide by MIC (AUC/MIC). Since only the unbound proportion of drug is pharmacologically active, an italicized prefix *f*, implying the free drug concentration, is often seen with these indices.

The PK/PD index that associated with specific antibiotic agent is determined from either the dose fractionation study in the rodent or the time-kill kinetic studies (Sy et al. 2016). The microbiological outcome, which is quantified by the change in log10 colony-forming unit (CFU), usually correlated very well with at least one of these MIC-

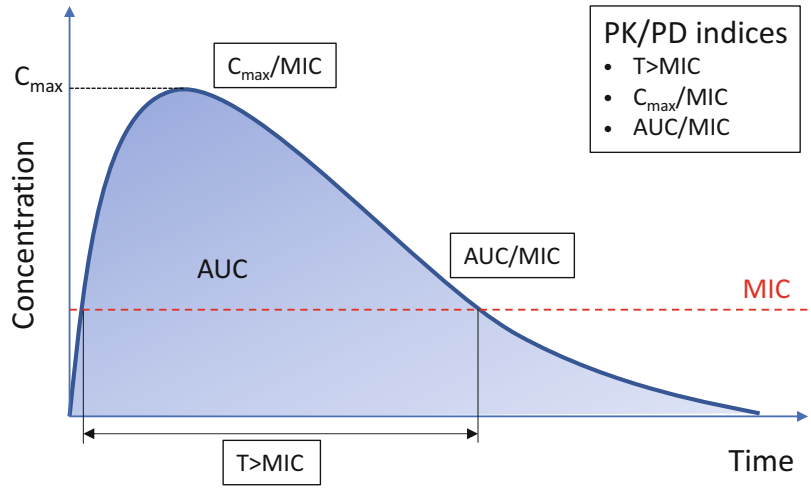

Fig. 2 Pharmacokinetic-pharmacodynamic indices as targets for achieving antimicrobial efficacy. T > MIC, the percentage of a dosing interval in which the drug concentration is above the MIC; C_{max}/MIC, the ratio of the maximal drug concentration to the MIC; AUC/MIC, the ratio of the area under the drug concentration-time curve to the MIC

based PD surrogate indices. If the PK/PD relationship is best characterized by T > MIC, implying time-dependent bacterial killing, i.e., once the free drug concentration is above the MIC, increasing the antibiotic concentration will not further increase the killing rate, but extending this period would ensure drug efficacy. One classic example of "time-dependent" killing is β-lactam antibiotics as the efficacy is enhanced with longer exposure times at which the free concentration maintained above MIC. In the case of antibiotics exhibiting concentration-dependent killing, the antimicrobial killing rate increases as the concentration of antibiotic increases. Therefore, the aim for this type of antibiotic is to attain the magnitude of fC_{max}/MIC ratio, which is proportional to the initial killing rate. The killing pattern of aminoglycosides correlates well with this ratio. For drugs which have concentration-independent killing but with persistent effects after drug exposure, their killing can typically be characterized by fAUC/MIC ratio. The bacterial killing rate for this type is related to both the duration when free drug concentration is above the MIC and the total exposure of the antibiotic. A good example that belongs to this group is vancomycin due to its extended postantibiotic effects that inhibit bacteria regrowth even free drug concentration dropped below MIC.

Time Course-Based Approaches

One of the limitations with the simple MIC-based approach is that the complex interactions between the host, the pathogen, and the drug itself are difficult to be captured by this static *in vitro* parameter. Different combinations of bacteria growth and killing kinetics in response of antimicrobial agent may lead to the same MIC in the end of incubation period. In addition, the MIC determined by twofold dilution is a relatively crude index, rather than an accurate estimation. Furthermore, the antibiotic concentration can remain constant during the time course of *in vitro* MIC measurement, or keep changing to mimic the dynamic change of free concentration at the actual target site *in vivo* . Therefore, another approach that provides more detailed PK/PD information to evaluate time course of bacterial response with dynamic exposure of the anti-infective agent is the *in vitro* time-kill assays.

The time-kill experiments can be either static (fixed antibiotic concentration) or dynamic (constantly changing antibiotic concentration). During static time-kill study, a sample was taken at each prespecified time, and bacterial colonies were counted after incubation. Although this experiment is simple, static studies are labor intensive as the drug concentration ranges from 0.25-fold up to 16-fold based on twofold dilution, and the duration of each experiment varies from 6 to 72 h (Sy et al. 2016). For dynamic time-kill study, the drug concentration in the medium is changing by

replacing with the fresh ones without antibiotic contained, rather than having the same medium throughout the experiment. The dynamic time-kill experiments are usually conducted via a hollow-fiber system where the model is continuously flushed with the fresh medium using a flow rate which is similar as the half-life of the investigated agent. Therefore, the *in vivo* drug kinetics can be simulated by this *in vitro* system.

Animal Models

The *in vitro* setting conditions, for either MIC determination or time-kill assays, are still highly distinct from the kinetic situation *in vivo* at the site of infection. Instead of aerobic and protein-free *in vitro* environment, the *in vivo* condition is rather anaerobic, acidic, and prone to protein binding (Levison 2004). Also, one of the drawbacks of hollow-fiber model is that the diffusion blockage may occur due to bacteria clusters adhering along the capillary walls, which may ultimately alter the flow rate in the system (Sy and Derendorf 2016). To overcome the limitation of *in vitro* PK/PD evaluation, such as lack of immune system and limited nutrients available, *in vivo* evaluation of PK/PD relationship is also feasible. One possibility would be to use animal models such as rats or mice.

Drug distribution at the target site are most commonly studied in thigh or lung infection models usually carried out in neutropenic rodents to avoid the variability of different immunity levels. Neutropenia can be induced by administration of cyclophosphamide (Zuluaga et al. 2006). Due to faster drug elimination in animals than humans, uranyl nitrate administration was performed before treatment to induce transient renal impairment, therefore delaying drug elimination in animals (Craig et al. 1991). PK samples were collected using microdialysis technique to measure the unbound drug concentration in the ISF at the target tissue. The bacterial density change from the starting inoculum was evaluated at the end of treatment period. These PD results were correlated with the PK/PD indices (i.e., $fT > MIC$, $fC_{max} > MIC$, and $fAUC/MIC$) in dose fractionation studies. Animal models provide a more pertinent approach to evaluate humanlike PK/PD; however, one of the biggest disadvantages is this procedure is not able to mimic human PK for drugs with extensive hepatic metabolism (Sy et al. 2016).

Model-Based Drug Development

Establishing PK/PD relationship using modeling and simulation is very critical for dosing regimen selection of antimicrobial agents, as the delineation of such relationship can greatly help selection of dosing regimen that has a high probability to overcome bacterial resistance and optimize clinical outcome (Drusano 2004). These model-based approaches provide useful information on the PK/PD index that best characterize the antimicrobial activity, as well as the translational value based on *in vitro* time-kill kinetics or animal PK data. In this section, we will discuss and illustrate the application of pharmacometrics in antimicrobial drug development. For MIC-based PK/PD indices, the dose and dosing interval can be determined through simulation of human PK and the desired target value of PK/PD index. When it comes to time-kill-based or animal PK/PD data, different dosing regimens can be evaluated via simulation of bacterial responses based on semi-mechanistic models.

Monte Carlo PK/PD Simulations

Given robust descriptive PK model and PD index determined from preclinical experiments, simulation of virtual clinical trials with different dosing regimens and targets can be done for dose optimization. Monte Carlo simulation is a computer-based mathematical technique that helps answer many "what if" questions before conducting expensive clinical trials in patient population (Roberts et al. 2011). Utilizing results from PK/PD simulation provides better confidence to the dosing regimen selected for clinical trials. The major components of Monte Carlo simulation include essential PK parameters and their corresponding interindividual variability, predefined antibiotic PD index, or parameters from semi-mechanistic PD models. For MIC-based approach, the simulated individual concentration-

time profile can be evaluated against the prespecified PK/PD index. If PK/PD model was used for simulation, optimal dose selection would be based on simulated data of bacterial response over time. However, simulation results may be invalid due to potential confounding factors or small sample size in PK data used for model development, or model development is based on total concentration rather than free concentration. In these situations, the simulation results should be interpreted with caution.

Probability of Target Attainment

For MIC-based approach, the percentage of the virtual subjects that achieve the target PK/PD index under certain dosing regimen can be computed from simulated individual PK profiles. This is defined as the probability of target attainment (PTA). PTA is usually determined from 1000 to 10,000 individual concentration-time profiles simulated from population PK model, considering the interindividual viability. Under each dosing regimen, the likelihood of achieving target attainment at a prespecified MIC value was calculated based on the distribution of PK/PD index (e.g., $fT > MIC$, fC_{max}/MIC, $fAUC/MIC$) from simulated PK profiles. This specific target is often associated with 1- or 2- log10 reduction of bacterial count from animal studies. After repeating the above likelihood calculation with a range of increasing MIC values, PTA and MIC can be plotted with x-axis as the MIC values and y-axis as the probability. This plot indicates the trend of less probability of successful antimicrobial achievement with increasing MIC. A probability greater or equal to 90% is usually accepted.

To illustrate the application of this approach, the example used here is from Singh and his colleagues (2017). PTA analysis of tigecycline was performed on traditional PK/PD target AUC/MIC >6.96 h and the new target of fAUC/MIC >2.05 h, accounting for atypical nonlinear plasma protein binding. The simulation was based on 10,000 individuals with body weight, creatinine clearance, and gender incorporated into the individual clearance. Individual AUC at steady state for dosing intervals ($AUC_{ss(0–24h)}$) was calculated from daily doses of 100, 150, 200, 250, and 300 mg intravenously. At each MIC level in a range of 0.064–64 mg/L, individual AUC/MIC ratio was calculated using each $AUC_{ss(0–24h)}$ divided by the corresponding MIC value. If the virtual subject has an AUC/MIC ratio greater than 6.96 h, the clinical outcome on this subject is considered as successful. Subsequently, the overall percentage of virtual subjects was computed at each MIC level under each dosing regimen (Fig. 3). Applying similar approach but combined with a protein binding model, fAUC/MIC ratio of each individual was calculated, and the percentage of virtual subjects achieving fAUC/MIC at least 2.05 h at different MICs with different tigecycline daily doses is also shown in Fig. 3.

If the target is based on AUC/MIC, a significant increase of target achievement is clearly shown with increasing daily dose. In contrast, dose increment does not significantly alter target attainment if it is based on fAUC/MIC. This finding implies the importance of using free concentration and exposure in PK/PD index for drug efficacy and the impact of protein binding on clinical breakpoints. PTA analysis was also used in this example to evaluate the effect of plasma protein binding on tigecycline clinical breakpoint selection. As Fig. 4 shows, the PTA analysis results at a daily dose of 100 mg; the clinical breakpoint for tigecycline against *E. coli* is 0.5 mg/L without consideration of protein binding but is 0.25 mg/L regarding the free AUC. Since clinical breakpoint is a very critical criterion to stratify patients into different susceptibility phenotypes, ignoring the protein binding could put more patients into risk of clinical failure.

Another approach to utilize Monte Carlo simulation results for clinical outcome prediction is to compute the expected PTA for a given microorganism population with a specific dosing regimen (Asin-Prieto et al. 2015; Mouton et al. 2005). This is the so-called cumulative fraction of response (CFR), which can be calculated using the previous PTA values and MIC distribution in the equation of $\left(\mathrm{CFR}(\%) = \sum_{i=1}^{n} \mathrm{PTA}_i \times F_i\right)$, where PTA_i is the PTA at specific MIC and F_i is the bacterial isolate frequency at that

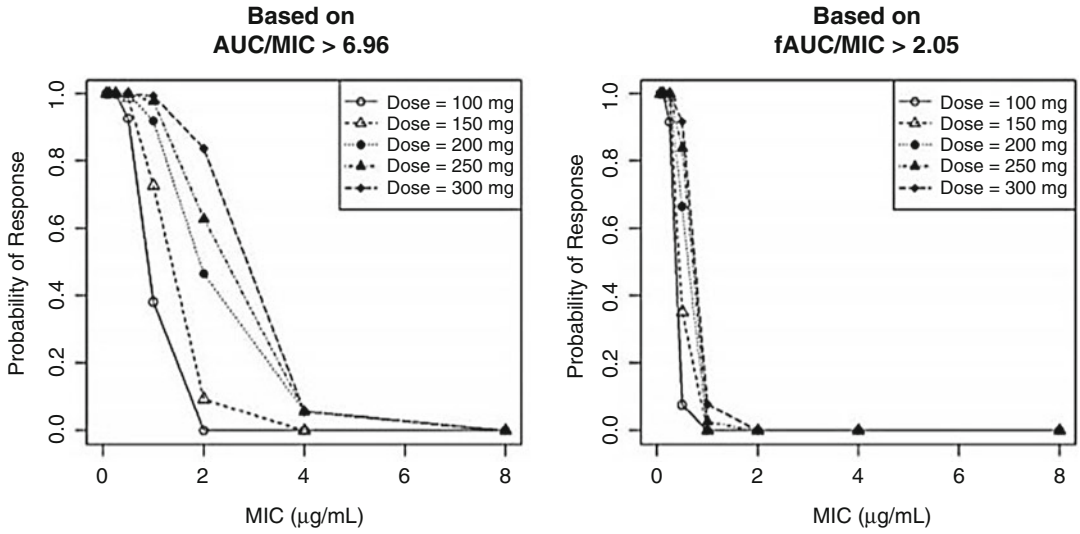

Fig. 3 Probability of target attainment as a function of minimum inhibitory concentration (MIC) of tigecycline against *Escherichia coli* at different doses with target AUC/MIC >6.96 h (left panel) and with target *f* AUC/MIC >2.05 h (right panel). (Image adapted from Singh et al. (2017) and used with permission)

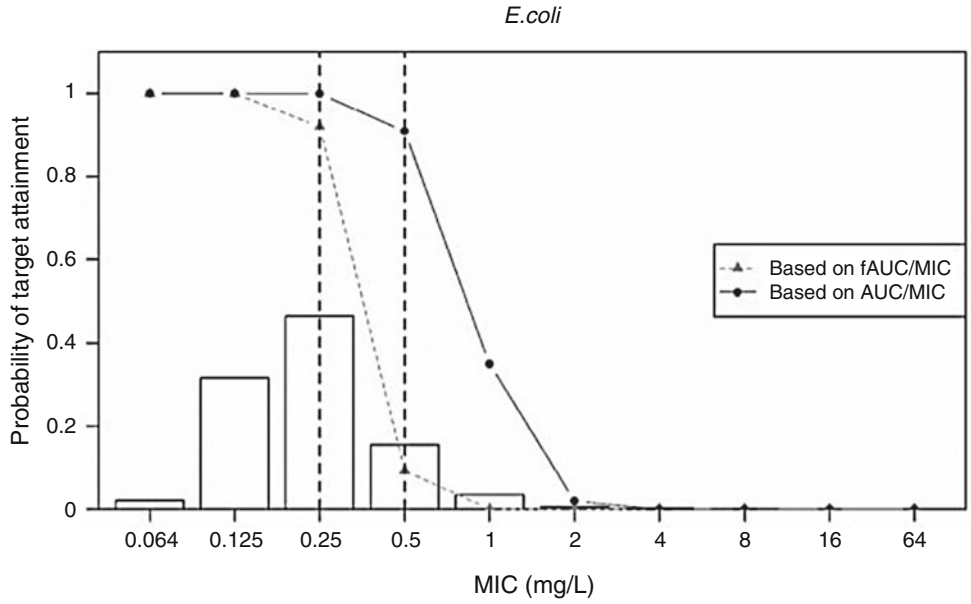

Fig. 4 Probability of target attainment at different minimum inhibitory concentrations (MICs) of tigecycline against *Escherichia coli* with total AUC/MIC (target 6.96 h) and ƒAUC/MIC (target 2.05 h). The distribution of MICs was obtained from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). (Image adapted from Singh et al. (2017) and used with permission)

corresponding MIC level. In this case, CFR is particularly useful when the exact pathogen susceptibility is unknown because the success of clinical outcome is predicted based on the whole population rather than a single MIC value. The population MIC distribution can be obtained from

European Committee on Antimicrobial Susceptibility Testing (EUCAST) or from a particular healthcare facility. It is important to notice that the pathogen susceptibility can vary between different locations and over time (Roberts et al. 2011; Asin-Prieto et al. 2015). Therefore, CFR is often specific as its calculation is based on the MIC distribution in a specific facility at a particular time.

Semi-mechanistic PK/PD Model

If the PD data is from time-kill curve experiments, semi-mechanistic PK/PD model is commonly used to establish the relationship between the bacterial colony population change and antimicrobial concentration over time. The information used for PD model development is often borrowed from static time-kill experiments, while the dynamic time-kill data provides additional information on PK/PD relationship and thus can be used for model validation. Rather than complicated mechanistic models considering the bacterial growth cycle, states of bacterial susceptibility, drug-receptor information, and the mechanisms of drug action, semi-mechanistic models are empirical models with simpler terms but still able to capture the bacterial response on antibiotics and monitor the development of bacterial resistance (Sy et al. 2016).

In the absence of antimicrobial intervention, the population number of bacteria in an inoculum over time represents the net result of bacterial natural self-replication and degradation (Jusko 1971):

$$\frac{\mathrm{dN}}{\mathrm{dt}} = k_{\mathrm{growth}} N - k_{\mathrm{death}} N \tag{16}$$

where N is the bacterial count and k_{growth} and k_{death} are the first-order rate constant for bacterial growth and death, respectively. This model assumes constant growth and death rate; however, it is difficult to separately define each constant as the observations are often based on $k_{\mathrm{net}} = k_{\mathrm{growth}} - k_{\mathrm{death}}$. Also notice that in both static and dynamic time-kill curve studies, bacteria grow exponentially but then reach a plateau or stationary level when the net growth is zero (Nielsen et al. 2011b). A logistic growth model (Tam et al. 2005) can better describe this self-limiting growth pattern:

$$\frac{\mathrm{dN}}{\mathrm{dt}} = k_{\mathrm{net}} \left(1 - \frac{N}{N_{\max}}\right) N \tag{17}$$

where $N_{\max}$ is the carrying capacity or the maximum population size in the system. From the analytical solution of Eq. 17, one important characteristic of this model is that bacteria population approaches the carrying capacity as time goes to infinity, i.e., $\left(\lim_{n\to\infty} N(t) = N_{\max}\right)$. In other words, bacterial population achieves stationary phase when N approaches $N_{\max}$.

When the *in vitro* system is exposed with antimicrobial agents, the drug effect can be incorporated into the logistic growth model such that:

$$\frac{\mathrm{dN}}{\mathrm{dt}} = k_{\mathrm{net}} \left(1 - \frac{N}{N_{\max}}\right) N - f_{\mathrm{kill}}(\mathrm{drug}) \tag{18}$$

where the f_{kill}(drug) is the drug effect (Nolting et al. 1996; Mouton and Vinks 2005; Liu et al. 2005; Treyaprasert et al. 2007), which is often represented by a sigmoid $E_{\max}$ or simple $E_{\max}$ model as

$$f_{\mathrm{kill}}(\mathrm{drug}) = \frac{E_{\max} C^{\gamma}}{\mathrm{EC_{50}}^{\gamma} + C^{\gamma}} N \tag{19}$$

where C is the drug concentration, $E_{\max}$ is the maximum killing effect, and $\mathrm{EC_{50}}$ is the concentration at which half of the maximum drug effect is achieved. The shape parameter γ, also known as the Hill factor, is 1 in simple $E_{\max}$ model.

Modification on Eq. 18, such as incorporating an adaptation or delay function, allows the logistic growth model to adapt to bacterial regrowth. With an adaptation function on $\mathrm{EC_{50}}$, the drug effect is modified as $\left(f_{\mathrm{kill}}(\mathrm{drug}) = \frac{E_{\max} C^{\gamma}}{(\alpha \bullet \mathrm{EC_{50}})^{\gamma} + C^{\gamma}} N\right)$, wherein $\alpha = 1 + \beta(1 - e^{-C\tau t})$ with β the maximal adaptation and τ the rate of adaptation factor (Tam et al. 2008). By using this adaptation function, the decline in the kill rate over time was successfully modeled. Subsequently, the model can well

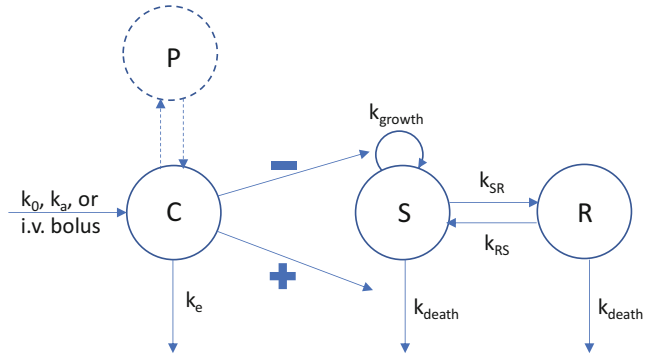

Fig. 5 Schematic illustration of the pharmacokinetic/pharmacodynamic model. *C* central compartment; *P* peripheral compartment; *S* proliferating and drug-sensitive bacteria; *R* resting and drug-insensitive bacteria; k_0 drug infusion rate constant; k_a drug absorption rate constant; k_e drug elimination rate constant; k_{growth} and k_{death} rate constants for multiplication and degradation of bacteria, respectively; k_{SR} rate constant for transformation from the growing, sensitive stage to the resting stage; k_{RS} rate constant for transformation from the resting stage to sensitive stage

predict the microbial responses to both gentamicin and amikacin. A delay term, usually expressed as $1 - e^{-\text{kt}}$, ranges between 0 and 1 from time zero to infinity. This function can be incorporated on the bacterial growth phase and/or anti-infective drug effect (Nolting et al. 1996; Liu et al. 2005; Treyaprasert et al. 2007). Treyaprasert et al. tried different types of delay function in PD model of azithromycin against four bacterial strains and found that incorporating delay term on both term can best describe the antibiotic response (Treyaprasert et al. 2007).

An alternative method to describe the self-limiting growth capacity is to implement the idea of bacterial phenotypic switching between susceptible (S) normally growing cells and persistent resting (R) cells with reduced growth rates (Balaban et al. 2004). In this case, the total number of bacteria would be the sum of both subpopulations (S + R) as it is illustrated in Fig. 5. In this model, assumptions were made as the majority of the bacteria in the early growth phase are in susceptible state and bacteria in persistent state do not respond to antimicrobials. As the bacteria population in the system increases, bacteria in growing stage are gradually transformed into a resting stage, leading to the stationary phase. The kinetic behavior of each subpopulation without antibiotic exposure can be described in the following equations:

$$\frac{\text{dS}}{\text{dt}} = k_{\text{growth}}S - k_{\text{death}}S - k_{\text{SR}}S + k_{\text{RS}}R \quad (20)$$

$$\frac{\text{dR}}{\text{dt}} = k_{\text{SR}}S - k_{\text{RS}}R - k_{\text{death}}R \quad (21)$$

where k_{SR} and k_{RS} are the transfer rates between susceptible population and resting population. Since persistent population is unlikely to return it back to susceptible state, thus the term k_{RS} can often be set as 0. The antimicrobial effect, with same sigmoid $E_{\max}$ model in Eq. 19, can either decrease bacterial growth rate or increase bacterial death rate and incorporated as an additive or proportional effect:

$$\frac{\text{dS}}{\text{dt}} = k_{\text{growth}}(1 - f(\text{drug}))S - k_{\text{death}}S - k_{\text{SR}}S + k_{\text{RS}}R \quad (22)$$

$$\frac{dS}{dt} = k_{growth}S - (k_{death} + f(drug))S - k_{SR}S + k_{RS}R \quad (23)$$

$$\frac{dS}{dt} = k_{growth}S - k_{death}(1 + f(drug))S - k_{SR}S + k_{RS}R \quad (24)$$

Nielsen et al. used this semi-mechanistic model to simultaneously fit *in vitro* static and dynamic time-kill data of *Streptococcus pyogenes* exposed to five different antibiotics (Nielsen et al. 2007, 2011b). The model was modified with an addition of an effect compartment to describe the time delay of drug effect. The investigators later on extended this model with a binding model implemented to describe the on and off adaptive resistance for gentamicin (Nielsen et al. 2011a). Simulation was performed on this model. The dose fractionation study indicated that the PK/PD indices can be identified from *in silico* predictions based on this semi-mechanistic PK/PD model. This study implied the power of applying PK/PD model derived from *in vitro* studies to describe the antimicrobial effect and select the optimal dosing regimen for clinical studies.

Models of Combination Therapies

Combination therapy, a drug intervention consisting of at least two therapeutic agents for the treatment of same condition, has gain popularity in recent years to overcome the emergence of bacterial resistance. The effect of combination therapy can also be characterized by logistic growth model utilizing Loewe additivity to evaluate synergistic effect of drug combination when each component has its intrinsic antimicrobial activity (Greco et al. 1995). Under the assumption that each therapeutic agent cannot interact with itself, the E_{max} model to evaluate the drug combination of two agents can be expressed as:

$$E_{max} = \frac{k_{max}\left(\frac{C_1}{\alpha_1 EC_{50,1}} + \frac{C_2}{\alpha_2 EC_{50,2}} + \frac{\gamma C_1 C_2}{\alpha_1 \alpha_2 EC_{50,1} EC_{50,2}}\right)^k}{1 + \left(\frac{C_1}{\alpha_1 EC_{50,1}} + \frac{C_2}{\alpha_2 EC_{50,2}} + \frac{\gamma C_1 C_2}{\alpha_1 \alpha_2 EC_{50,1} EC_{50,2}}\right)^k} \quad (25)$$

where k_{max} is the initial killing rate, α_i refers to the same adaptation function defined previously, and γ indicated the Loewe synergism ($\gamma > 0$) or Loewe antagonism ($\gamma < 0$). A semi-mechanistic PK/PD model incorporating Loewe additivity has been used to successfully describe the combination effect of vertilmicin (an aminoglycoside) and ceftazidime (a β-lactam) against *Pseudomonas aeruginosa* (Zhuang et al. 2015). In this case, both vertilmicin and ceftazidime have antimicrobial activities with their own mechanism of action. Characterizing the potential synergistic effect in PK/PD modeling and simulation would assist dose selection of this therapeutic combination.

When it comes to the combination of β-lactam (BL) and β-lactamase inhibitor (BLI), such as avibactam and aztreonam, ceftazidime and avibactam, and meropenem and vaborbactam, the BLI has limited intrinsic antimicrobial activity but restores the antimicrobial activity of BL, leading to an enhanced spectrum of activity with this type of drug combination. Given that the microorganism's susceptibility to BL enhanced with increasing concentration of BLI and the degradation of BL also depends on bacterial density, it is reasonable to incorporate a drug degradation model into the semi-mechanistic PK/PD model. Sy et al. have developed such models for ceftazidime/avibactam combination to predict the exposure-response behavior of both agents (Sy et al. 2017, 2018). The schematic representation of the model structure is illustrated in Fig. 6.

Similar as other semi-mechanistic models of logistic growth equation, the antimicrobial effect of BL can be described in a sigmoidal E_{max} model. However, instead of using a simple EC_{50} parameter for drug potency, a bi-exponential function of BLI concentration is applied in Eq. 19 such that BL EC_{50} shifts toward lower values with increasing BLI concentrations. Therefore, the function of drug effect has been modified to:

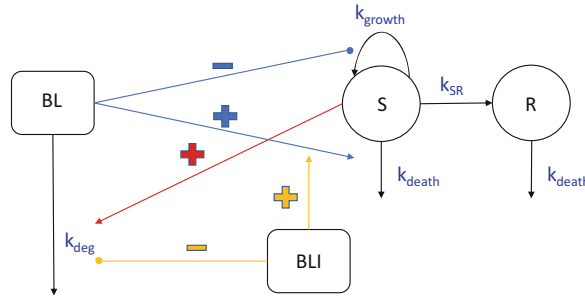

Fig. 6 Schematic representation of the combined effects of β-lactam antibiotic (BL) and β-lactamase inhibitor (BLI) on the growth and killing of pathogens in a closed system including bacterial density-dependent BL degradation. (Image adapted from Sy et al. (2018) and used with permission)

$$f_{\text{kill}}(\text{drug}) = \frac{E_{\max} \bullet BL^{\gamma}}{\left(A\mathrm{e}^{-\alpha \bullet \text{BLI}} + B\mathrm{e}^{-\beta \bullet \text{BLI}}\right)^{\gamma} + BL^{\gamma}} N \tag{26}$$

where A and B are constants with their sum determines the initial value of EC_{50} when BLI is absent. α and β are the exponents that describe the relationship of BLI concentration and BL potency. The bi-exponential model was used since BL MIC drops rapidly from monotherapy to combination therapy with even the lowest BLI concentration, and then BL MIC continues decreasing but in a slower pace as BLI concentration increases. Given that MIC correlated to EC_{50} in the logistic growth model (Mouton and Vinks 2005), this bi-exponentially decreasing function is suitable to model the mechanism that BLI restores BL susceptibility to bacteria. As previously discussed, a delay function can also be implemented on the growth and/or kill term in this semi-mechanistic model to modulate the initial decline of bacteria and delay in later regrowth in the presence of BLI.

During the time-kill studied by Sy et al., degradation of both BL and BLI was also monitored. A drastic degradation rate was seen when bacterial population is high in the system and BL degraded in a concentration-dependent manner of BLI. Hence, a saturable Michaelis-Menten-type equation was applied to BL degradation model with degradation rate depending on bacteria population as well as the concentration of both BL and BLI:

$$\frac{\text{dBL}}{\text{dt}} = -\frac{\text{Deg}_{\max} \bullet N^{\varphi}}{{K_m}^{\varphi} + N^{\varphi}} \left(1 - \frac{\text{BLI}}{\text{IC}_{50} + \text{BLI}}\right) \text{BL} \tag{27}$$

where $\text{Deg}_{\max}$ is the maximum degradation rate constant, K_m is the bacteria density at which degradation rate is half of the maximum value, φ is the Hill coefficient that determines the shape of the function, and IC_{50} is the BLI concentration that yields 50% decrease of the BL degradation rate.

By applying this semi-mechanistic model, considering bacteria-mediated BL degradation and inhibition of degradation of BLI, Sy et al. were able to develop the PK/PD model from static time-kill data to simultaneously describe the dynamic change of multidrug resistant *Pseudomonas aeruginosa*, ceftazidime degradation, and the inhibition effect from avibactam (Sy et al. 2018). This model was further validated using dynamic time-kill data as well as data from animal models. By incorporating the mechanism of drug resistance, the model was able to give more detailed prediction of the bacterial dynamics in response to BL/BLI combination. It has the potential to expedite the BL/BLI combination drug development by confident simulation of clinical trials.

Special Population

Since most of the time, the initial PK model development is based on data from healthy adult volunteers in Phase 1. Drug PK may not be the same for patients or special populations such as obese population, geriatric population, pediatric

population, etc. due to the different ontogeny, physiology, and pathophysiology conditions in these special populations. Elderly patients are particularly subject to drug toxicity including antibiotics due to their diminished physiology reserve and the frequent polypharmacy, therefore requiring dose adjustment of some antimicrobial agents (Benson 2017). For pediatric population, allometric extrapolation of clearance can give reasonable pediatric dose for similar exposure in children if using a drug-specific allometric exponent rather than using the fixed exponent of 0.75 (Mahmood 2007). It was also found that an age-dependent exponent in allometric scaling model can well predict the first-in-children dose (Mahmood et al. 2014). Dosing in obesity is also complicated not only due to the physiological alternation and comorbidity but also the lack of standardized measure of creatinine clearance and the variability of types of body weight used for dose adjustment (Meng et al. 2017). Renal impairment has a big impact on the PK of drugs which are extensively eliminated by kidney (≥30%). Particularly, for patients with end-stage renal disease (ESRD, renal function is less than 10% of the normal capacity), whether dose needs to be adjusted and how the dose should be adjusted becomes a critical issue as ESRD patients also routinely receive hemodialysis to assist drug removal. Pharmacometrics is critically valuable for dose optimization in special population. The dosing finding can be based on both approaches as we discussed previously: (1) PTA based on PK modeling and simulation and PK/PD indices and (2) simulation based on semi-mechanistic PK/PD model.

An example is illustrated here on the application of PK/PD approach for gentamicin dosing strategy in ESRD patients receiving hemodialysis (Zhuang et al. 2016). Similar approach can also be utilized for dose adjustment in other special populations. A one-compartment model was able to describe gentamicin PK adequately. An additional clearance during hemodialysis was incorporated into the model as $CL_{TOT} = CL_{NR} + CL_R + HEMO \cdot CL_{HD}$ such that the total clearance is the sum of nonrenal clearance, renal clearance, and hemodialysis clearance when dialysis is on (HEMO is an indicator with value 1 when hemodialysis is on and 0 when hemodialysis is off). PD model was developed based on data from *in vitro* static and dynamic time-kill studies against three bacterial strains. An adaptive factor was incorporated onto EC_{50} as previously described.

Based on this semi-mechanistic PK/PD model, Monte Carlo simulation was performed with two dosing regimens: (i) 120 mg after hemodialysis, which is the recommendation from gentamicin label, and (ii) 240 mg 1 h before hemodialysis, which has been used in several literatures. The concentration-time profiles for 1000 subjects are showing in Fig. 7 (upper) with the lines indicating mean values and shaded bands implying the 50% (dark) and 95% CI (light). The bacterial killing over time in ESRD patients undergoing both dosing regimens was also predicted (Fig. 7 lower). Simulation results suggested that PTA of fC_{max}/MIC > 8 are 10% and 100% for the first and second dosing regimen, respectively. Since gentamicin toxicity is associated with the trough concentration, therefore PTA of fC_{trough}/MIC < 2 was also calculated for both dose regimens, yielding 80% and 25%, respectively. PTA results implied the first dosing regimen has better safety but lower efficacy than the second. Predictions of bacterial response from the semi-mechanistic PK/PD model suggested that the second dosing regimen is only slightly better than the first one with both dosing regimens displaying bacterial density reduction of $>1 \times 10^2$. Therefore, the author concludes that the first dosing regimen provides a well-balanced benefit/risk profile than the second regimen in ESRD patients.

Modifications of the Method

Pharmacometrics has become a very important component in drug development to maximize the clinical potential of drugs. During the recent 30 years, PK/PD approaches have been rapidly evolving and widely spread in academia, pharmaceutical industry, and regulatory agency. The principles and applications presented here illustrate

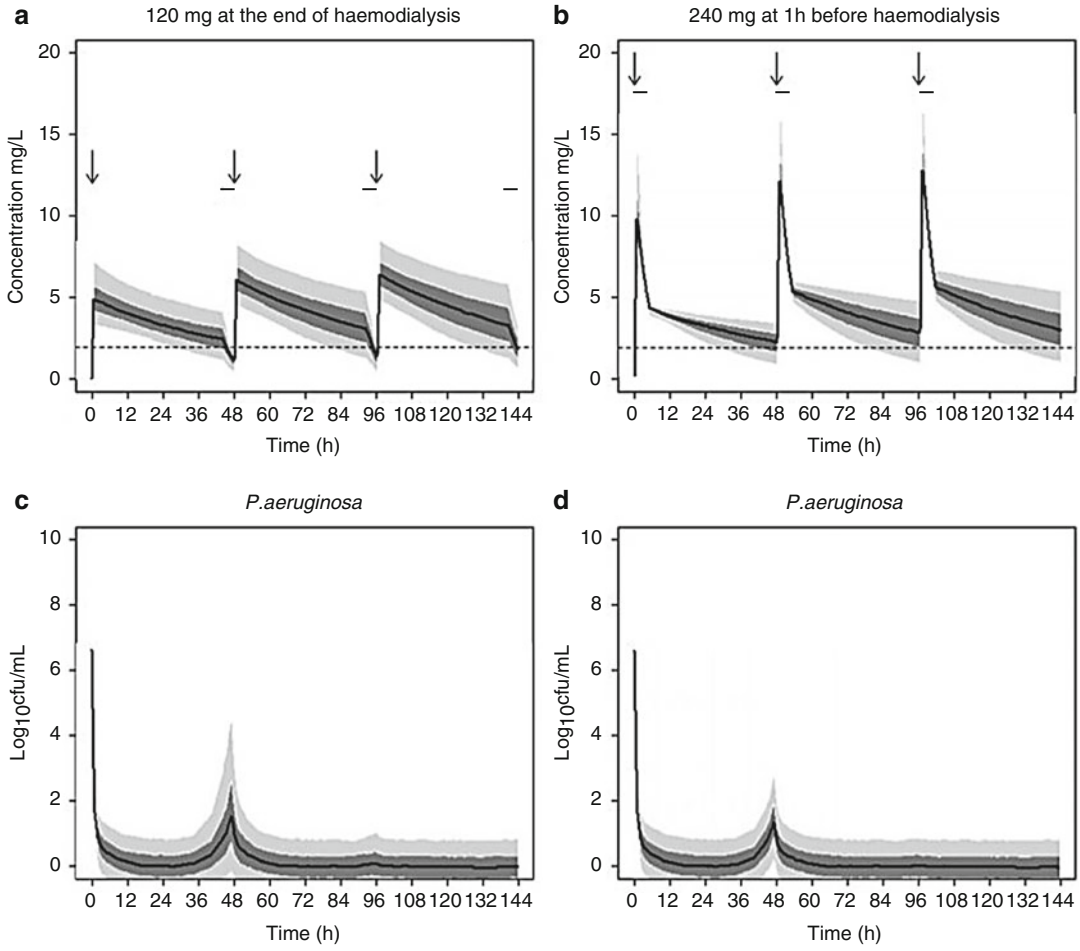

Fig. 7 Prediction of antibacterial activity of gentamicin against *P. aeruginosa* under two dosing regimens. (**a**) Concentration-time profile with 120 mg at the end of hemodialysis; (**b**) concentration-time profile with 240 mg 1 h before hemodialysis; (**c**) bacterial response-time profile with 120 mg at the end of hemodialysis; (**d**) bacterial response-time profile with 240 mg 1 h before hemodialysis. Dashed lines represent the safety threshold (2 mg/L). Arrows represent dosing time. Tick error bars represent the hemodialysis time interval. (Image adapted from Zhuang et al. (2016) and used with permission)

the opportunities in model-informed drug development. Books from Ette and Williams (2007) and Schmidt and Derendorf (2014) provide excellent overview on the basic principles of pharmacometrics and numerous examples of its applications to expedite successful drug development.

FDA and EMA issued guidelines on population PK (FDA 1999; EMA 2007) and other related topics such as pediatrics (EMA 2006), exposure-response relationships (FDA 2003), or QTc interval prolongation (FDA 2005). Both agencies have emphasized the power of PK/PD approach in effective data leveraging and risk/benefit balancing for rational dose selection and response prediction. Currently, FDA is conducting model-informed drug development pilot program to facilitate decision-making and improve trial success probability for drug development.

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