Pharmacokinetic-Pharmacodynamic Modeling of Antibacterial Drugs
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Pharmacokinetic-pharmacodynamic (PKPD) modeling and simulation has evolved as an important tool for rational drug development and drug use, where developed models characterize both the typical trends in the data and quantify the variability in relationships between dose, concentration, and desired effects and side effects. In parallel, rapid emergence of antibiotic-resistant bacteria imposes new challenges on modern health care. Models that can characterize bacterial growth, bacterial killing by antibiotics and immune system, and selection of resistance can provide valuable information on the interactions between antibiotics, bacteria, and host. Simulations from developed models allow for outcome predictions of untested scenarios, improved study designs, and optimized dosing regimens. Today, much quantitative information on antibiotic PKPD is thrown away by summarizing data into variables with limited possibilities for extrapolation to different dosing regimens and study populations. In vitro

**Abstract**—Pharmacokinetic-pharmacodynamic (PKPD) modeling and simulation has evolved as an important tool for rational drug development and drug use, where developed models characterize both the typical trends in the data and quantify the variability in relationships between dose, concentration, and desired effects and side effects. In parallel, rapid emergence of antibiotic-resistant bacteria imposes new challenges on modern health care. Models that can characterize bacterial growth, bacterial killing by antibiotics and immune system, and selection of resistance can provide valuable information on the interactions between antibiotics, bacteria, and host. Simulations from developed models allow for outcome predictions of untested scenarios, improved study designs, and optimized dosing regimens. Today, much quantitative information on antibiotic PKPD is thrown away by summarizing data into variables with limited possibilities for extrapolation to different dosing regimens and study populations. In vitro

**ABBREVIATIONS:** AUC, area under the concentration-time curve; CART, classification and regression tree; CFU, colony-forming units; CL, clearance; CLCR, creatinine clearance; C_{max}, maximum concentration during a dosing interval; E_{max}, maximum effect; f_u, fraction unbound; IIV, interindividual variability; IOV, interoccasion variability; LOD, limit of detection; MDR, multidrug resistant; MIC, minimum inhibitory concentration; MSW, mutant selection window; MTT, mean transit time; MU, million units; PK, pharmacokinetics; PD, pharmacodynamics; PTA, probability of target attainment; t_{1/2}, half-life; T-MIC, time above MIC; V_d, volume of distribution; VPC, visual predictive check.
studies allow for flexible study designs and valuable information on time courses of antibiotic drug action. Such experiments have formed the basis for development of a variety of PKPD models that primarily differ in how antibiotic drug exposure induces amplification of resistant bacteria. The models have shown promise for efficacy predictions in patients, but few PKPD models describe time courses of antibiotic drug effects in animals and patients. We promote more extensive use of modeling and simulation to speed up development of new antibiotics and promising antibiotic drug combinations. This review summarizes the value of PKPD modeling and provides an overview of the characteristics of available PKPD models of antibiotics based on in vitro, animal, and patient data.

I. Introduction

The world is facing an alarming scenario in which we are rapidly losing treatment options due to resistance against multiple currently available antibiotics. At the same time, the pharmaceutical industry is losing interest in developing new antibiotics because of their expected limited return on the investment. The demand for new antibiotics and optimized treatments makes quantitative approaches to predictions of bacterial killing a valuable asset for rational drug development and drug usage. In pharmacokinetic-pharmacodynamic (PKPD) modeling, the relationship between dose, concentration, and desired effects and side effects is described and quantitated.

Pharmacometrics has been defined as “the science of developing and applying mathematical and statistical methods to characterize, understand, and predict a drug’s pharmacokinetic and pharmacodynamic behavior” (Ette and Williams, 2007). Pharmacometrics has been promoted as a methodology to rationalize and inform drug development and is widely appreciated by the pharmaceutical industry, academia, and regulators in general. Developed models have been described as “powerful platforms that regulators may use to compile and analyze data in order to support approval and labeling” (Manolis and Herold, 2011). Pharmacometrics is a bridging discipline, as the field includes pharmaceutical sciences, clinical pharmacology, medicine, computational science, programming, and statistics.

PKPD models are typically illustrated by compartments and schematic boxes, including representations of transfer of amounts and relationships between the compartments. The change in amounts in the compartments over time are typically described by differential equations (Fig. 1) with the parameters of the equations being estimated based on available data through an iterative search. Once a model has been built, based on different type of information for the system of interest, it can be used in computer simulation to describe, explain, and investigate different scenarios (Mould and Upton, 2012). PKPD modeling and simulation can be a tool in the selection of dosing regimens that result in the antibiotic concentration-time profile that will achieve the desired outcome. However, we believe PKPD modeling is an underused approach in the development and improvement of antibiotic dosing regimens. To date, much focus has been on summary endpoints for drug exposure and efficacy. Models that can integrate the time courses of the relationships between dose and concentration, between PK and antibacterial effect and side effects, between PK and resistance development, as well as the host response to the infection would be very valuable for rational drug development.

The purposes of modeling are to describe the general trend and variability in the observed data at hand but also to better understand underlying mechanisms of drug action and the interaction on the physiologic system; to predict tested and untested doses and dosing regimens; to simulate outcomes of new studies; and to be a tool for design of new studies. A model should be built for its purpose and capture the crucial aspects of the processes. PKPD modeling and simulation is currently applied in all larger pharmaceutical companies to quantitatively characterize the observed drug responses and mechanisms of drug action, as well as variability, and to support decision making. For the last three decades, PKPD modeling has also been applied to improve dosing regimens of approved drugs and to individualize therapy.

An established PKPD model can be very valuable for early predictions of a drug effect, where the information on outcome is limited. On the basis of an available model that possesses a generic structure with model parameters that describe the underlying system, limited data are needed to describe the drug’s effect on the system. An example of such a model that has been shown to be applicable across drugs and various phases of drug development is a PKPD model for myelosuppression (Friberg et al., 2002; Karlsson et al., 2005). A similar mechanism-based model could be useful for predicting the time-course of bacteria growth and kill in patients, based on in vitro and/or in vivo information. Much of a model structure can be shared across bacterial strains, and parameters describing growth characteristics in the absence of drug should be drug-independent. It follows that the requirement of experimental data on candidate drugs can be relatively sparse and still allow for the drug-effect parameters to be successfully estimated.

This review provides an overview of available pharmacokinetic-pharmacodynamic models of antibacterial drugs that are based on in vitro, animal, and patient data, and we discuss why and how such models can be more efficiently applied to optimize the use of currently available antibiotics and to facilitate
development of new antibiotics. The focus of the review will be on data-driven models where parameters are estimated, and we will also provide a general background on pharmacokinetics, pharmacodynamics, and PKPD modeling and simulation (pharmacometrics).

II. Pharmacokinetics, Pharmacokinetics-Pharmacodynamics, and Drug Development

While PK has been defined as “how the body handles the drug,” PD has been defined as “how the drug affects the body” (Rowland and Tozer, 2011). PK and PD are key components in modern drug development. Once PK and PKPD are characterized, the concentration that leads to the desired effect and limited side effects can be identified, and the dosing regimen that will result in the target concentration range can be computed.

A. Pharmacokinetics

PK is a central part of clinical pharmacology and pharmacometrics. PK describes the relationship between drug dosing and the drug concentration-time profile in the body. The drug concentration is typically determined in plasma, and the change over time \(C\) can in the simplest case be approximated to decline from an initial concentration \(C_0\) with time \(t\) by an exponential function

\[
C(t) = C_0 \times e^{-k_e \times t}
\]

Equation 1 represents a one-compartment PK model, i.e., one distributional phase is sufficient to describe the decline in concentration. The elimination rate constant, \(k_e\), is of first order, i.e., it has the unit of “per time” (e.g., hour\(^{-1}\)), which means that the elimination at any given time point is proportional to the concentration or amount remaining in the system. The corresponding differential equation can be written as

\[
\frac{dC}{dt} = -k_e \times C
\]

where \(k_e\) is the parameter to be estimated based on the data, e.g., through an iterative process in a designated software. The half-life \((t_{1/2})\) is inversely related to \(k_e\) \(t_{1/2} = \ln(2)/k_e\). From eqs. 1 and 2, it follows that once \(k_e\) is known, the drug concentration can be predicted at any time point for a given \(C_0\). The shape of the PK profile will however depend on the dose, the administration route and formulation, the dosing frequency, as well as the disposition of the drug (Fig. 2).

\(k_e\) is determined by the apparent volume of distribution \((V_d)\) as well as clearance \((CL)\) that describes the elimination capacity, which is typically governed by liver and kidney function. For a drug with immediate distribution and a \(CL\) value independent of concentration, \(k_e\) can be described as

\[
k_e = \frac{CL}{V_d}
\]

Often, the drug disposition is more complex because the distribution is not immediate and the concentration-time course will be better described by two or more compartments (Fig. 1). The differential equations for a two-compartment model can be written as

\[
\frac{dA_c}{dt} = -\frac{CL}{V_c} \times A_c - \frac{Q}{V_c} \times A_c + \frac{Q}{V_p} \times A_p
\]

\[
\frac{dA_p}{dt} = -\frac{Q}{V_p} \times A_p + \frac{Q}{V_c} \times A_c
\]

where \(A_c\) and \(A_p\) are the amounts in the central and peripheral compartments, and \(V_c\) and \(V_p\) are the corresponding volumes of distribution. \(Q\) represents the intercompartmental clearance. An intravenously administered dose would be given into the central compartment.

The total exposure is often described as the area under the concentration-time curve (AUC). AUC is obtained by integrating the drug concentration-time profile and can also be computed as the systemically available dose over \(CL\). The bioavailability, \(F\), determines the fraction of an extravascular dose that reaches the systemic circulation and is thereby a measure of the extent of absorption. The rate of absorption is often characterized by a first-order rate constant, \(k_a\). For a detailed introduction to PK and PD, see, e.g., the textbook by Rowland and Tozer (2011) and the tutorial on PK modeling by Mould and Upton (2013).

Many drugs are bound to proteins (primarily to albumin and/or \(\alpha\)-1-acid-glycoprotein) in plasma, and the binding in both plasma and tissue will have consequences on the drug disposition. It is the free, unbound concentration of the drug that is able to distribute, to be eliminated, and to interact with
receptor and other effector sites. Therefore, the free fraction ($f_u$) rather than total (free + bound) should be used to drive a PKPD model.

B. Pharmacodynamics

PD describes the relationship between concentration and both the wanted and unwanted effects. In PKPD modeling, a link between the PK and its influence on an effect variable is established by a mathematical function (see example in Fig. 1). The effect variable may be a measurement such as glucose or blood pressure, a composite score such as an outcome describing success or failure or a time to an event or cure. The effect measurement at any given time is determined by a function of its value without drug ($E_0$) and the drug concentration ($C$). Frequently the mathematical function describing the PKPD relationship is a sigmoidal $E_{max}$ model (eq. 6).

$$E(t) = E_0 + \frac{E_{max} \times C(t)^\gamma}{EC_{50} + C(t)^\gamma}$$

where $E_{max}$ is the maximum effect that can be achieved by the drug in the investigated system and $EC_{50}$ is the drug concentration that results in half of the maximum effect. $EC_{50}$ is inversely related to the potency. $\gamma$ is the Hill or sigmoidicity factor that determines the steepness of the relationship but is in many cases not statistically significant from 1. As $\gamma$ increases, the relationship becomes steeper and will eventually approach a step-function or an all-or-none effect. One reason for the popularity of the $E_{max}$ model is that the function asymptotes to an upper limit of stimulation or inhibition by a drug on a system. However, often there are situations when sufficiently high concentrations cannot be achieved to estimate $E_{max}$, and simplifications can be made where fewer parameters are estimated. When $C < EC_{50}$, the $E_{max}$ model collapses to a linear model ($\gamma = 1$) or a power function ($\gamma \neq 1$) with coefficient $Slope$ as shown in eq. 7.

$$E(t) = E_0 + Slope \times C(t)^\gamma$$

The underlying $E_0$ is not always constant over the study period. For example, the effect variable may vary because of an underlying disease, such as fluctuations in glucose in the event of diabetes or a diurnal rhythm in blood pressure. The observed effect is hence the sum of the underlying effect variable in the absence of drug and the influence of the drug on the effect variable. The model complexity can increase with increased availability of data and knowledge of the underlying system. For example, there may be feedback mechanisms that regulate the measured variable, such as the influence of insulin on glucose levels. In addition, delays between concentration and effect are frequently observed. When delays between concentration and effect are frequently observed. A delay could be due to slow distribution to the effect site, (unmeasured) active metabolite formation, turnover of responses, signal transduction, and other mechanisms that result in a shift in time from the drug interaction until the effect is observed.

Often data are not continuous but categorical (e.g., cure/not cure, or none, mild, moderate, and severe) or the analysis variable is time-to-event data. In those
cases logistic regression models and survival analysis, respectively, are applied to describe the probability of the events. The principle for evaluating PKPD relationships however is often the same as for continuous type data. The relationship between concentration and the logit of the probability of the event is typically modeled as a linear or \( E_{\text{max}} \) function. For a dichotomous longitudinal variable the probability of an outcome \( (P_1) \) may be estimated based on eqs. 8 and 9

\[
L(t) = E_0 + \text{Slope} \times C(t)
\]

\[
P_1(t) = \frac{e^{L(t)}}{1 + e^{L(t)}}
\]

where \( L(t) \) and \( E_0 \) are the total and underlying effects on the logistic scale, respectively (Ette and Williams, 2007).

C. Population Pharmacokinetics and Pharmacokinetic-Pharmacodynamic Modeling

As indicated in the introduction, population PK and PKPD models are frequently developed and applied to characterize drug concentration and effects over time, and to optimize dosing regimens. A population model typically includes 1) a structural model describing the typical concentration-time and/or effect variable-time profiles in the population, 2) a statistical model quantifying and separating different types of variability, and, 3) a covariate model.

The variability between patients (interindividual variability, IIV), between occasions (interoccasion, IOV), and the residual error can be quantified in a population-PK(PD) model. For example, variability can often be considerably higher in patients than in healthy volunteers. The residual error includes measurement error in time and magnitude, intraindividual variability within an occasion, model misspecification, etc. The IIV (and IOV) are most often assumed to be derived from a parametric log-normal distribution, with the difference in the individual value of the parameter \( (P_i) \) and the population parameter value \( (P) \) described by \( \eta \) (eq. 10).

\[
P_i = P \times e^{\eta}
\]

\( P \) represents a parameter such as \( CL \) or \( EC_{50} \); \( \eta \) is generally assumed to arise from a normal distribution with a mean of zero and an estimated variance. In PD, variability in parameters such as the underlying baseline \( E_0 \) may be better described with an additive \( \eta \) or more skewed distributions (Petersson et al., 2009). Parameters may also be assumed to arise from a non-parametric distribution using support points and an associated probability (Jelliffe, 1991).

It should also be acknowledged that PK and PD may change within a patient from one day to another and because of change in the disease (see section VI.A.1). Systematic changes in parameters may, however, be difficult to capture unless studies are designed for that purpose. Variability day-to-day or between occasions often appears as if it is random nature, and IOV should be estimated in the modeling to not bias parameter estimates (Karlsson and Sheiner, 1993). In addition, the larger the IOV is in relation to IIV, the less is the value of feedback adaptation based on a measurement variable (e.g., plasma drug concentration) and a population model. In therapeutic drug monitoring (TDM) or target concentration intervention (TCI) (Holford, 1999), a patient’s PK parameters are determined based on an available population-PK model, the patient’s characteristics and dosing history, and a limited number of drug concentration measurements. On the basis of the estimated subject’s parameters and a target concentration (or target concentration range), an individualized dose is computed for the next dosing interval. The same methodology can be extended to biomarkers.

Another goal with population modeling is to search for covariates that can explain at least part of the variability. As an example, creatinine clearance (CL\textsubscript{CR}) is often a significant covariate for CL of renally excreted drugs, and incorporation of a relationship between this covariate and CL will likely reduce the unexplained variability between patients. CL\textsubscript{CR} can thereby provide guidance in the individualization and choice of dose so that patients with a low CL\textsubscript{CR} receive a lower dose in accordance with their reduced capacity to eliminate the drug. For PD variables, severity of disease, study differences, sex, and previous treatment are common covariates. The relative decrease in the IIV upon inclusion of the covariate relationship can be computed to investigate the importance of the covariate in explaining variability (Matthews et al., 2004).

A population-PK(PD) model can serve as a tool for compiling different types of available information. As new findings and study data come along, these can be integrated into the model structure and/or parameters can be re-estimated to improve and extend the predictive capacity. A pharmacometric model can therefore be viewed as a pool of existing information that is continuously being updated during drug development.

D. Simulations

When a PKPD model has been developed, the outcome of different inputs to the model, i.e., different dosing regimens, can be investigated and predicted. Since the administration schedule and route of administration affects the shape of the concentration-time profile (Fig. 2), the predicted effect will also be dependent on the input dosing regimen. Because of the variability components, different individuals will have different PK profiles and different concentration-effect relationships. The outcome in a population can therefore be seen as a distribution of effects in the population. The variability in the effect will depend on the
variability in the population-PK and -PKPD parameters, which are estimated in a population model as described above. Based on a developed model, the variability in an outcome can be simulated.

Within the area of population PKPD modeling, stochastic (Monte Carlo) simulations have been applied for over 3 decades. In stochastic simulations, samples from a variability distribution of $\eta$ values are randomly drawn to create individual parameter values (eq. 10) for patients in a simulated study population (Bonate, 2011). The individual parameters will construct the concentration- and effect-time profiles for each simulated patient, and the results can be informative on the likely outcome for a group of patients given a certain dosing regimen.

In 1981, D’Argenio performed simulations for trial design (D’Argenio, 1981), and in 1989, Sheiner and Beal presented the application of stochastic simulations for a dose-ranging study to explore different assumptions and scenarios (Sheiner et al., 1989). The concept of clinical trial simulation was extended in the 1990s to include different aspects of the trial design, the aim being to integrate relevant information before conducting the trial and thereby reduce the risk of a trial to fail because of poor design. The history and power of clinical trial simulation have been nicely reviewed by Holford et al. (2000, 2010). Simulations can also be applied retrospectively to explain why trials failed and learn from them, for example, why a trial did not show a statistically significant effect despite a significant concentration-effect relationship (Friberg et al., 2009). Simulations are also valuable for model evaluation (Yano et al., 2001) by comparing the trends and distribution of the observed data to the model-predicted distribution of simulated data (see section VII.C).

E. Modeling and Simulation in Drug Development

Clinical drug development has traditionally been divided into three phases. In Phase I trials escalating doses of single and multiple doses are administered, typically to healthy volunteers, and the goal is to determine PK and side effects. In Phase II the aim is to confirm signs of efficacy and evaluate different doses and schedules. In Phase III efficacy of the drug is compared with currently used therapy or placebo. Clinical development has become more extensive and complex over the last decade as regulatory demands increase, and consequently development has become more expensive, although development times have not increased (DiMasi et al., 2010).

With increasing cost, fewer trials can be performed, and thereby, there will be fewer chances to show efficacy in trials for a single drug. It is therefore vital to study informative dosing regimens to be able to draw the right conclusions from the trials. With modeling and simulation, competing trial designs can be explored, including innovative designs, maximizing informativeness, and study power. Drop out can be handled adequately, resulting in limited bias in conclusions.

Full integration of the pharmacometrics approach into drug development procedures has been called “model-based” drug development, and it has been suggested that modeling and simulation can reduce attrition rates, improve on chances to reach scientific goals, and provide support for early go/no-go decisions (Mould and Upton, 2012). Pharmacometrics has been recognized by the FDA in the Critical Path Initiative document (Woodcock and Woosley, 2008), which aims to make drug development faster at a lower cost. There is in general a growing emphasis on the use of modeling and simulation also in regulatory decision making. Examples where PKPD modeling has supported regulatory decisions are in the selection of dosing regimens, approval of regimens that have not been directly studied in clinical trials, and use of such data to support a single pivotal (Mould and Upton, 2012).

III. Pharmacokinetics-Pharmacodynamics of Antibiotics

The PKPD of antibiotics differ from other drugs in that the targeted species is different from its host, and an ideal antibiotic would not directly affect the host and cause side effects (although the gut flora is frequently affected by antibiotic therapy). For the relationship between drug concentration and efficacy in terms of bacterial killing the PD definition “how the drug affects the body” would be more adequately phrased as “how the drug affects the bacteria.”

The first example of the fact that the dosing schedule can affect the outcome, showing the relevance of PKPD relationships for antibiotics, was demonstrated already in 1950 when Eagle et al. demonstrated antibacterial activity to be time-dependent for penicillin and concentration-dependent for streptomycin (Eagle et al., 1950). The PKPD relationships of antibiotics are now routinely being searched for to help establish dosing guidelines. However, the relationships for dose optimization are still being based on summary variables despite that there is increased knowledge on bacteria dynamics and the limitation of using summary variables in predicting development of drug resistance. In this section, we will present and discuss some of the commonly used PD variables for antibiotics—the minimum inhibitory concentration (MIC), PK/PD indices, and clinical breakpoints—and provide some reflections on PKPD of antibiotics in drug development.

A. Minimum Inhibitory Concentration

Minimum inhibitory concentration, MIC, has been the major parameter for quantifying bacterial susceptibility against an antibiotic. The MIC test is relatively
Broth dilution methods use liquid medium in which a specified bacterial inoculum \([5 \times 10^5\) colony-forming units (CFU/ml)\] is exposed to a constant antibiotic concentration during an incubation period of 16–20 hours. The MIC is defined as the lowest drug concentration that during these conditions completely inhibits visible growth of the microorganism. The static antibiotic concentrations chosen for MIC determinations, using dilution techniques, are typically based on 2-fold dilutions (e.g., 0.5, 1, 2, and 4 concentration units). Depending on the total volume used the method is either termed macrodilution (1–2 ml) or micro-dilution (\(\leq 500 \mu l\)). For agar diffusion methods, an agar plate is inoculated and the antibiotic diffuses from a disk or a strip into the agar. One example of an agar diffusion method is the E-test, in which the bacterial growth around a strip impregnated with an exponential gradient of the antibiotic, is assessed after incubation for 24 hours. The E-test is less labor-intensive than the broth dilution technique; however, its use is restricted to those antibiotics that are supplied by the E-test manufacturer.

The MIC value is a measure of the net effect on growth and antibiotic-induced bacterial killing over the incubation period, evaluated at a “snapshot” time and at a fixed concentration, and the dilution approach induces an up to 2-fold error compared with the actual MIC. MIC is hence a crude, monodimensional threshold value that neglects measurement error and any dynamic changes in growth and susceptibility over the studied time period. In addition, MIC tests are based on ocular inspection and are thus associated with a subjective error. Therefore, MIC is not a good PD parameter to characterize the concentration-effect relationships. Another problem is that the MIC is often thought of as a threshold value for drug effect, i.e., the MIC value is interpreted as: there is no bacterial killing at all when concentrations are below its value.

**B. Pharmacokinetic/Pharmacodynamic Indices**

During the last two decades PKPD relationships of antibiotics have been classified into three different PK/PD indices based on a summary measure of drug exposure that is linked to the MIC of the bacteria (Vogelman et al., 1988; Craig, 1998). The PK/PD index approach has become the gold standard for evaluating PKPD of antibiotics and to guide establishment of dosing regimens, and the approach has been applied to a wide range of infection types, patient groups, and antibiotics. However, the PK/PD indices have several drawbacks associated with assumptions made when neglecting information on the time-course of PK and PD.

The notation of the three PK/PD indices have been standardized (Mouton et al., 2005) into \(fT_{\text{MIC}}, fC_{\text{max}}/\text{MIC}\) and \(fT_{>\text{MIC}}\). AUC is the area under the concentration-time curve, \(C_{\text{max}}\) is the highest concentration reached (the peak), and \(T_{>\text{MIC}}\) is the cumulative percentage of a 24-hour period that the concentration is above MIC. The prefix, \(f\), is introduced to indicate that the free, unbound fraction of the drug was used in the calculations. If there are no subscripts indicating a time interval, it is assumed that the calculations of AUC and \(T_{>\text{MIC}}\) were based on a 24-hour interval at pharmacokinetic steady-state conditions.

The best PK/PD index for a certain drug-bacteria combination is determined by plotting the value of an efficacy endpoint (typically log10 CFU/ml after 24 hours of treatment) versus the magnitude of each of the three PK/PD indices (see Fig. 3). The best PK/PD index for the drug-bacteria combination is determined by fitting a sigmoidal \(E_{\text{max}}\) model (eq. 6, redefined with PK/PD index–related notation in eq. 11) to the summary PD endpoint and the three PK/PD indices. The index with the best fit (highest coefficient of determination, \(R^2\)) is chosen.

\[
E = E_0 - \frac{PD_{\text{max}} \times X^\gamma}{EX_{50} + X^\gamma} \tag{11}
\]

\(E\) is the summary PD endpoint, and \(E_0\) is the effect representing the value of the PD endpoint without drug treatment (i.e., the value of the summary endpoint when the PK/PD index is 0). Because the bacteria population grows during the 24 hours in the absence of antibiotics, \(E_0\) is several log10 higher than the starting inocula (Fig. 3). \(X\) is one of the three PK/PD indices as defined above, \(PD_{\text{max}}\) is the maximum effect (in relation to \(E_0\)) indicated by the plateau where increased exposures result in no further kill. \(EX_{50}\) is the magnitude of \(X\) that is needed to achieve 50% of \(PD_{\text{max}}\), and \(\gamma\) is the sigmoidicity factor. AUC and \(C_{\text{max}}\), and sometimes \(T_{>\text{MIC}}\), are often highly correlated, making it important to have data from several different dosing regimens to be able to distinguish between them. Dose-fractionation studies are performed in animals for this purpose (see section V). The specific value of the PK/PD index that is needed to result in a pronounced reduction in CFU/ml, compared with untreated animals, is determined from the estimated relationship and defined as a target magnitude of the PK/PD index. Common targets are 2- or 3-log kill, but the PK/PD index magnitude at 90% of \(E_{\text{max}}\) has also been applied as target. The steepness of the relationship (i.e., the value of \(\gamma\)) determines the difference in the magnitude of the PK/PD index resulting in this value (Fig 3). It has been suggested that the magnitude of the PK/PD index determined in mice is similar to that needed for clinical effectiveness (Ambrose et al., 2007), and a correspondence has also been found when predicting PK/PD indices using a
A mechanism-based model developed on in vitro data (Nielsen et al., 2011a). The PK/PD index determined from nonclinical studies is used today in combination with clinical information to determine the optimal dose and dosing regimens as discussed below and in section VI.B.

Although the application of the PK/PD index approach may appear to be relatively intuitive for optimization of dosing regimens, it needs to be acknowledged that the indices are simplifications of the PKPD relationship. Figure 3 illustrates that despite well-performed studies, none of the indices results in a perfect fit. Different dosing regimens resulting in the same PK/PD index value may be related to a multiple log variability in CFU at 24 hours, a variability that is typically not considered when the PK/PD index targets are determined. The figure also shows that two or more of the PK/PD indices can show similar $R^2$ values, despite fundamental differences in their implications when used in the selection of optimal dosing regimens.

All indices rely on MIC, and drawbacks described above for MIC are thus propagated into the PK/PD indices, and it is also assumed that MIC will stay

![Fig. 3. Illustration of relationships for *P. aeruginosa* for thigh (left) and lung (right) between log$_{10}$ CFU and each of the three PK/PD indices. Each symbol represents one observation data point. The lines represent the fit of sigmoid $E_{\text{max}}$ functions, and the variance of the regression ($R^2$) is presented for each PK/PD index. The dashed lines indicate the mean bacterial burden at start of treatment. The variability for a given magnitude of a PK/PD index illustrates the uncertainty in the endpoint extracted from these types of experiments. Reproduced from Dudhani RV, Turnidge JD, Coulthard K, Milne RW, Rayner CR, Li J, and Nation RL (2010) Elucidation of the pharmacokinetic/pharmacodynamic determinant of colistin activity against *Pseudomonas aeruginosa* in murine thigh and lung infection models. *Antimicrob Agents Chemother* 54:1117–1124, with permission from American Society for Microbiology.](image-url)
constant during a treatment period. Furthermore, AUC dependence indicates independence on rate of drug administration, e.g., that a high spike concentration with a very rapid elimination will result in the same bacterial killing as a continuous infusion as long as the total AUC is the same. A $C_{\text{max}}$/MIC relationship indicates that the effect is only dependent on the highest concentration achieved and independent of drug half-life. A $C_{\text{max}}$-dependent drug is thus very sensitive to infusion length, with a 15-minute infusion resulting in a lower effect than a bolus injection (Fig. 2). $T_{\text{>MIC}}$ dependence indicates that the antibacterial effect is at its maximum just above MIC and that there is no further killing by increasing the concentration. It has been recognized however that the maximum is reached at concentrations around four to five times MIC (Craig, 1998). Most often there is no “correct” PK/PD index, although approximations may work well for some substances. Karlsson et al. have suggested a general model for time-dissociated effects where AUC-dependent and time-dependent relationships are specific cases (Karlsson et al., 1998). However, as already emphasized, the best understanding of the PKPD relationship is gained by modeling the full time-course of bacterial growth and killing.

A PK/PD index also relies on that the summary PK variables in the experiments are correctly determined and it is generally assumed that a PKPD index identified in one population (or in preclinical studies) can be directly applied on another patient population. However, it has been illustrated that the best fitted PK/PD index can depend on the half-life of the antibiotic (Nielsen et al., 2011a) and thus differs among patient populations with varying elimination capacity. For drugs like the $\beta$-lactams where the efficacy generally have been found to be correlated to $T_{\text{>MIC}}$, the best PK/PD index shifts toward AUC/MIC dependence as the half-life increase, as seen in patients with a reduced renal function e.g., elderly or neonates. Similarly, for an AUC/MIC dependent drug a decrease in half-life will lead to a shift into a $T_{\text{>MIC}}$ relationship, requiring more frequent dosing. It should also be acknowledged that the shape of the concentration-time profile can differ in the target tissue compared with plasma. The different shapes of the $E_{\text{max}}$ models shown for thigh and lung in Fig. 3 may be due at least partly to differences in distribution.

As indicated, more informative measures are needed when extrapolating to different patient populations, when several drugs are used in combination and when considering resistance development. Determination and application of PK/PD indices in the context of animal data and in relation to clinical outcomes are further described in sections V.B and VI.B, respectively.

C. Probability of Target Attainment

In 2001, Drusano and colleagues introduced stochastic (Monte Carlo) simulations in the field of antibiotics by integrating a population-PK model with the PK/PD index defining microbiological susceptibility (Drusano et al., 2001). The individual PK parameters simulated from the population PK model will create, based on a given dosing regimen, concentration-time profiles from which a PK/PD index magnitude is computed for each individual. Based on the distribution of individual PK/PD index magnitudes, the likelihood of achieving a certain target or therapeutic outcome in the population is predicted from the distribution.

In the example by Drusano et al. (2001) on evernimicin, different types of $fAUC_{24}$/MIC target values were evaluated based on the experimental findings in a neutropenic murine thigh infection model: stasis at 24 hours, 1- to 3-log killing, and 90% of maximal killing effect. The probabilities of achieving the targets were compared for two dose levels on different bacteria types. During the last decade, this Monte Carlo simulation methodology has been used to determine the currently used dosing regimens for numerous antibiotics, bacteria types, and indications.

To investigate the probability of target attainment (PTA), a large population ($n = 1000–10,000$) is simulated and the proportion of simulated subjects above an identified target (e.g., the $fAUC_{24}$/MIC required for 2-$\log_{10}$ kill determined from animal experiments, i.e., an in vivo static effect) is computed for a range of MIC values and dosing regimens. The proportion of subjects with values of the PK/PD index above the target constitutes the PTA at each MIC. PTA is plotted or tabulated as a function of MIC, given a dosing regimen and a PK/PD index target value (Fig. 4A), preferably with information added regarding an observed MIC distribution (Owens et al., 2005). It is still under debate which probability level, e.g., 90, 95, or 99%, should be regarded as acceptable to ensure a high probability of success for microbiological cure (Mouton et al., 2012). Because the choice of target is subjective in nature, plotting the relationship between the “best” PK/PD index and the MIC (Fig. 4B) also has been suggested. The reader/user then can determine the PTA based on any target size of the PK/PD index and MIC for a given dosing regimen.

Differences in PK parameter estimates (typical or variability estimates) between healthy volunteers and patients, or between different patient populations, will influence the shape of the PTA curve. Initial PK studies are often performed in small, homogeneous populations. Increasing the PK variability in the simulations to better reflect a true patient population will result in a less steep PTA curve, with lower target attainment in the region of interest (Mouton et al., 2012). In general, simulations of PTA are performed using a fixed PD target, assuming that the PK/PD target is determined without error or uncertainty and that the target is the same for all patient populations and bacterial strains. Recently, an example was...
presented where variability in the PD target was considered (MacGowan et al., 2009). The authors included a bacterial strain-to-strain variation in the PK/PD index required for efficacy in the simulations. As seen for increased variability in PK, inclusion of variability in the PD target resulted in a less steep PTA curve, and in the example shown, the change in the PTA was large enough to influence the susceptibility breakpoint determination. Furthermore, the PK (and PD) parameters used in the simulations are associated with an uncertainty, which propagates into the simulation and thus should be accounted for and visualized in the PTA analysis (Nielsen et al., 2012).

D. Clinical Breakpoints

The original definition of breakpoints was aimed at finding the MIC value that separates a wild-type distribution of microorganisms into susceptible and antibiotic-resistant phenotypes. Today, that categorization, based on MIC distributions, is named “epidemiologic cut-off value,” whereas “clinical breakpoints” consider the shape of the PK profile. Clinical breakpoints are set by determining, based on observations in experimental and clinical studies, a pharmacodynamic target that distinguishes between patients who are likely or unlikely to respond to the treatment. These clinical breakpoints are aimed at providing guidance to clinicians in their daily work of treatment selection for specific species of bacteria. The clinical breakpoints can be derived by the following: 1) use of typical PK parameters and a cut-off value for the MIC (the deterministic approach), or 2) account for the variability in PK between patients and in the MIC of the targeted pathogen population (probabilistic approach, “PKPD breakpoints”).

The introduction of Monte Carlo simulations (see section III.C) into the antimicrobial field led research groups and organizations to make intensive efforts to review clinical PK data and establish suitable clinical breakpoints under in vivo conditions where PKPD is a key component. Since 2002, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has applied Monte Carlo simulations to set clinical breakpoints (Mouton et al., 2012). In the determination of breakpoints, Monte Carlo simulations are performed for the currently used dosing regimen based on the following: 1) a population-PK model, ideally developed from the patient population of interest; 2) the unbound fraction ($f_u$) of the drug in patients; 3) the PK/PD index magnitude needed to achieve the target determined from preclinical and/or clinical studies; and 4) the MIC distributions for a certain bacteria species, which is valuable to display in the PTA plot (Fig. 4). The clinical breakpoint is the MIC that separates low and high probability of cure based on the achieved PK/PD index value for a given dosing regimen but should preferably not divide the wild-type MIC distribution. Bacteria with MICs that result in PK/PD index values that are lower than the pharmacodynamic target for an expected exposure should be considered resistant.

E. Pharmacokinetics-Pharmacodynamics and the Resistance Problem

Bacteria are prone to adapt to the environment and develop resistance upon antibiotic drug exposure. The resistance can be acquired by mutations in the chromosome but also through horizontal gene transfer where foreign genetic material is transmitted between
bacteria in free form, in plasmids, and by bacteriophages (Aleksun and Levy, 2007). Several mechanisms for antibiotic resistance have been described, including drug efflux where the drug is pumped out of the bacteria, altered target sites affecting the affinity of the drug for its target site, and deactivation of the drug by different enzymes (Austin and Anderson, 1999). If the drug is pumped out of the bacteria or if the drug is deactivated by enzymes, it is likely that increasing the concentration further by increasing the dose might help to overcome the drug resistance. On the other hand, if the target site is drastically altered, increasing the drug concentration is not likely to have much of an effect (Austin and Anderson, 1999). In some cases multiple mutations are necessary to acquire high level resistance. For the fluoroquinolones several mechanisms of resistance have been identified, e.g., including alteration in genes coding for the drug target (DNA gyrase and topoisomerase IV) or genes regulating drug efflux. Individually, the alterations result in low level resistance; however, when combined a high level resistance is acquired (Marcusson et al., 2009). On the other hand, for rifampicin a single point mutation in the gene coding for the drug target (beta subunit of RNA polymerase) have been associated with high level resistance in *Escherichia coli* (Jin and Gross, 1988).

For ciprofloxacin, irreversible resistance frequently emerges during treatment, and even very low concentrations (1/10 to 1/230 times MIC of wild-type bacteria) have been shown to be sufficient to select for resistant bacteria (Gullberg et al., 2011). Once a mutation has occurred, the chance that the bacteria revert to being sensitive is low, and it is more likely that a new mutation is acquired to increase the fitness (Andersson and Hughes, 2010). During aminoglycoside exposure, bacteria often become refractory to the antibiotic action because a phenotypic resistance develops that is reversible upon drug removal (Barclay et al., 1992; Mohamed et al., 2012). Because of its transient nature the phenomenon of adaptive resistance is difficult to study. For the aminoglycosides, the mechanism is thought to involve an increased drug efflux through the MexXY-OprM efflux pump (Hocquet et al., 2003). The adaptive resistance of gentamicin is illustrated in Fig. 6, and adaptive resistance is further discussed in section IV.B.4.d. Because PK/PD indices are static variables, they cannot characterize such dynamic changes in the sensitivity over a treatment period.

There exists both experimental and clinical evidence that the choice of dosing schedule influences resistance development (Drusano, 2004; Ambrose et al., 2007). The relationship between exposure and resistance selection has been described by an inverted U-shaped function, and a mutant selection window (MSW) has been suggested (Tam et al., 2007), i.e., the selection for resistance starts at a certain concentration, but at higher concentrations, the population with a reduced sensitivity is killed as well. High concentrations are consequently needed to prevent amplification of resistant bacteria (Jumbe et al., 2003).

Mouton et al. (2011) have highlighted several priorities to combat resistance where PKPD is the key tool in improving the understanding of how to overcome and prevent resistance development. Some suggestions include increased doses to suppress resistant bacteria, shorter courses of therapy to reduce unnecessary use, and usage of antibiotic drug combinations. They also suggest re-evaluation of the dose regimens and indications of available antibiotics, based on new information on PKPD relationships, and improvement in the approval process by more efficient application of PKPD principles. However, in our view, to accomplish this efficiently, there is a need to move away from summary PKPD variables such as the PK/PD indices and MSW. In future developments of dosing schedules for antibiotic treatments, there should be at least two goals as follows: 1) efficient killing of susceptible bacteria and 2) prevention of evolution of drug resistance.

### F. Drug Development of Antibiotics

Despite the alarming scenario of increasing prevalence of resistance, the interest of pharmaceutical companies in developing antibacterial drugs has subsided dramatically, and most Big Pharma companies have abandoned the area because of relatively low return on investment and perception of burdensome regulatory environment. This is in spite of the relatively high success rate for infectious diseases and the fact that antibiotics rarely fail in clinical development for lack of efficacy (DiMasi et al., 2010). Efficacy endpoints for antibiotics are often clearly defined, but as the cure rate in antibiotic trials is relatively high, it is difficult for new antibiotics to show superiority. Sample-size calculations are therefore most often linked to estimation of noninferiority margins, although there is an uncertainty in how to determine the no-treatment effect, as the adequacy of using historical data has been questioned. In the new European Medicines Agency guideline for antimicrobials, the agency promotes trial sponsors to explore alternative and emerging methods for estimating the no-treatment effect and recommends pharmacometric approaches and thorough investigation of PKPD relationships based on in vitro, animal, and clinical data (European Medicines Agency, 2012). It is also stated in the guideline that “if the PK/PD relationship for an agent is very clear and the analyses are convincing it may be possible to omit clinical dose finding studies.” In a recent article, innovative trial designs are suggested for comparative studies of new agents for antibiotic-resistant bacteria (Infectious Diseases Society of America, 2012). The authors promote adaptive trial designs that consider PKPD knowledge, and pharmacometric approaches should be considered for evaluation of organism-specific outcomes.
Because the speed of antibiotic drug development has slowed down, there is a dramatic shortage of new antibiotic molecules in the clinical pipeline (Theuretzbacher, 2012). Twenty new antibiotics, of which few are from new antibiotic classes, were launched between 2000 and 2011 (Butler and Cooper, 2011). There are currently approximately 40 compounds in clinical development, but only a few of these have potential to act on multidrug resistant (MDR) gram negative bacteria (Butler and Cooper, 2011). To increase the enthusiasm for focusing development on antibiotics against MDR bacteria, there is a need for new approaches. One way could be to focus trials on patients infected by MDR pathogens, although such trials will include fewer patients. Alemayehu et al. (2012) have suggested the implementation of a gradual approval process where the initial approval is based on one robust nonrandomized study with supporting information based on PKPD from nonclinical studies and modeling and simulation. This proposal implies that much of the understanding of PKPD, including the characterization of resistance development, can indeed be gained from experimental systems. In addition, more efficient use of clinical pharmacology data can limit the number of trial subjects, as advocated in orphan drug development (Schmidt et al., 2008; Bashaw et al., 2011; Bassetti et al., 2011).

IV. Pharmacokinetic-Pharmacodynamic Modeling of In Vitro Data

A. Experimental In Vitro Systems

Various experimental in vitro systems have been developed to complement in vivo studies in the characterization of the PKPD of antibiotics. The in vitro studies are generally easier to perform, more cost effective, and allow for greater flexibility in the study design than the in vivo alternatives. In the in vitro studies, a start inoculum of a bacterial strain is exposed to an antibacterial agent and the amount of bacteria is assessed over time. Such experiments allow for a study design that covers the full effective concentration range and thereby supports a detailed characterization of the PKPD relationship. Modeling provides a tool to combine information from different types of experiments, creating a cohesive quantitative mathematical description of the growth and killing kinetics of the bacteria, and as outlined in the introduction, such models can be used to optimize future in vitro and in vivo studies. When used in combination with modeling, in vitro experiments can help to minimize the use of experimental animals and reduce the size of clinical trials, advantageous both for ethical and economic reasons. One disadvantage with in vitro studies is that growth and killing behavior might differ between the in vitro and in vivo situations. For instance, the killing as assessed in vitro usually only represents the antibacterial effect imposed by the drug, whereas in the in vivo situation, the antibacterial effect by the host immune system can be investigated.

An experimental setup similar to that used for macro-broth dilution MIC determination (section III. A) is used for time-kill curve experiments, but here the bacterial count is followed over time with frequent sampling. The drug exposure in these experiments may be static, as in the MIC experiments, but it can also be modified fairly easily to allow for a dynamic change in the concentration-time profile of the antibacterial agents.

A wide variety of experimental setups have been described in the literature over the last few decades. Here we will only focus on the experimental in vitro systems most commonly used in combination with mathematical PKPD modeling. There is no uniform classification or terminology when it comes to the different experimental in vitro systems. In a recent comprehensive review of the different in vitro systems, Gloede et al. (2010) classified the different in vitro systems according to whether the drug concentration is constant or changing and whether there is a bacterial loss from the system or not. In accordance with this, we divide the systems according to static versus dynamic systems as well as open versus closed systems. We use the terms “static” and “dynamic” systems depending on whether there is any exchange of media in the culture vessel or not. If there is an exchange, we further divide the dynamic systems according to working principle as follows: 1) systems based on dilution or 2) systems based on diffusion or dialysis (Grasso, 1985; Mueller et al., 2004; Gloede et al., 2010). We avoid the term static drug concentrations, because even in a static experimental setup, the drug concentration does not necessarily need to be constant since spontaneous drug degradation and/or time-dependent binding of the drug might occur (Bergan et al., 1980; Karvonen et al., 2011). Furthermore, the use of a static system does not mean a controlled static environment. The growth and killing of bacteria might affect the availability of essential nutrients and/or produce potentially toxic waste products. Here we use the term in vitro systems rather than in vitro models, the reason being that we want to restrict the term models to the context of mathematical models. For similar reasons, the term dynamic systems is used instead of the commonly used term in vitro kinetic models.

1. Static Systems. The static system represents the simplest experimental in vitro setup for PKPD characterization of antibiotics. With their simplicity, a large number of experiments can be performed, covering the full concentration-effect range. Therefore, data from the static system often form the fundamental knowledge regarding the relationship between drug exposure and efficacy. The system consists of a single culture vessel, usually a tube or a flask, containing the growth...
media, the bacteria, and the antibiotics. The total volume used is typically between 10 and 125 ml, with a working volume of 4–50 ml. The culture vessels are incubated at 35 or 37°C, and a shaker water bath could be used to ensure homogenous mixing and temperature. The system allows for samples to be drawn repeatedly during the incubation period. The incubation period in these experiments is rather short in general, usually \( \leq 24 \) hours, although longer experiments are sometimes performed and there are examples where the drug-containing medium was replaced with fresh drug-containing medium after 24 hours (Nicasio et al., 2012).

2. Dynamic Systems. In the dynamic system the drug concentration changes over time. The dynamic system is generally more time and labor consuming than the static system, but it makes it possible to simulate human PK in vitro. The dynamic system is also valuable when it comes to studying time-dependent PKPD characteristics, such as in the presence of a time delay before the bacteria resume growth after an intermittent antibiotic exposure, or, in the presence of adaptive resistance.

\( \text{i. Dilation methods.} \) In the dilution system, fresh antibiotic-free medium is added to the culture vessel during the experiment. This can be performed with or without a corresponding removal of medium from the culture flask, i.e., either by replacing or adding new medium. The addition can be made in a continuous or in a stepwise fashion.

In the simplest dilution systems, the dilution is accomplished by adding fresh medium in a stepwise fashion (Nishida et al., 1976; Delacher et al., 2000), manually or under computer control (Barclay et al., 1992). In 1996, Nolting et al. (1996) developed a system where the antibiotic-containing medium from the culture vessel was withdrawn at regular intervals by a syringe needle equipped with sterile filters and replaced with fresh antibiotic-free medium.

The stepwise dilution system is simple, but continuous dilution of medium resembles more closely the in vivo drug clearance process. In the original continuous dilution setup (Grasso et al., 1978), the system consists of a culture vessel (an Erlenmeyer flask) containing medium, bacteria, and antibiotics. This vessel is connected to a peristaltic pump, which pumps fresh medium into the culture flask from a connected reservoir. The built-up pressure in the vessel results in a corresponding outflow of waste medium, thus keeping the volume of fluid in the culture vessel at a constant level. In an alternative setup, the pump is instead used to remove medium from the culture vessel, with a corresponding inflow of fresh medium from the connected reservoir by the negative pressure that builds up in the vessel (Lowdin et al., 1996). Samples for viable count and drug concentration can be drawn repeatedly from the waste outlet (Grasso et al., 1978) or from a side arm of the culture flask (Lowdin et al., 1996). The system resembles the kinetics in a one-compartment model with a first-order elimination rate constant determined by the flow rate divided by the volume of medium in the culture vessel. The antibiotic can be added directly to the culture vessel or to the diluting medium to resemble a bolus dose or a continuous infusion, respectively. The setup can be modified to also include a first-order absorption process (Grasso et al., 1978) and/or to simulate a two-compartment disposition pharmacokinetic profile (Murakawa et al., 1980).

\( \text{ii. Closed Systems.} \) The system described by Grasso et al. (1978) is an open system in the sense that both drug and bacteria are diluted when fresh medium is added, and the dilution of bacteria should be taken into account in the interpretation of the results. To compensate for the bacterial dilution, a mathematical correction factor has been suggested. This factor is determined based on the differences in the growth and killing kinetics in the time-kill curves obtained following similar drug exposures in the static as well as the dynamic dilution systems (Keil and Wiedemann, 1995). Nevertheless, several assumptions are associated with the use of these correction factors and a more advantageous approach is to include the outflow of bacteria as a bacterial elimination rate in the PKPD modeling (Okusanya et al., 2011).

\( \text{b. Diffusion/dialysis methods.} \) The apparatus in the diffusion/dialysis method consists of two compartments, a central and a peripheral compartment, separated by a membrane. As in the dilution system, the drug is added to the central compartment and fresh medium is pumped through this compartment to reduce drug concentration. The drug has to diffuse through a membrane to reach the bacteria that are contained in a separate peripheral compartment. There is an exchange of drug, nutrients, and waste products between the two compartments by a bidirectional dialysis process. However, the bacteria are restricted to the peripheral compartment because of size. Several experimental setups and membrane types have been described, including systems with artificial or natural membranes (Gloeckle et al., 2010). With the single-membrane system (Bonapace et al., 2002), a slow equilibration process is obtained generally resulting in two-compartment
kinetics in the peripheral compartment (Zinner et al., 1981).

c. Hollow fiber system. The hollow fiber system consists of multiple dialysis capillaries collected together as one dialysis unit. These systems have a high, efficient surface area-to-volume ratio, providing for a rapid equilibration of the drug concentration between the central and peripheral compartment, with the kinetics in the peripheral compartment resembling the kinetics in the central compartment (Campion et al., 2005; Zinner et al., 2008). This technique is gaining in popularity, and hollow fiber reactors are commercially available. Zinner et al. (1981) described a hollow fiber system for studying PKPD of antibiotics, which has been developed further by others (Blaser et al., 1987; Lister et al., 1998; Ba et al., 2001; Gumbo et al., 2004). These systems consist of a bundle of artificial capillaries with the luminal space forming the central compartment. The capillaries consist of hollow fibers (e.g., polysulfone, polypropylene, or polyethylene fibers), which are semipermeable in the sense that the drug, nutrients, and waste products can diffuse over the membrane, but the bacteria cannot, i.e., they resemble a closed system. The capillaries are connected to a pump, and fresh medium is pumped through the fibers, with the flow rate and the volume of the in vitro system determining the elimination rate of the drug. The capillaries are placed in an outer peripheral vessel containing the bacteria. The concentration gradient across the membranes becomes the driving force for diffusion of the drug into this extracapillary bacterial compartment. The medium in the peripheral compartment is renewed by diffusion from the central compartment. Sampling ports are available in the bioreactor, and sampling is allowed in the central as well as the peripheral, extracapillary compartment. Constant infusion, intravenous bolus or oral dosing is mimicked by introducing the drug in the diluent reservoir, the central compartment, or an extra precentral absorption compartment, respectively (Blaser et al., 1987). Computer-controlled syringe pumps could be used to administer the antibiotic into the central reservoir according to the desired dosing schedule (Gumbo et al., 2004; Drusano et al., 2010). Because the hollow fiber system is a closed system, it is less prone to problems with contamination, and its one clear advantage is that the experiment can be extended over a longer time period, sometimes as long as several weeks (Drusano et al., 2010). The closed system is also an advantage when working with biohazardous organisms.

3. Experimental Conduct.

a. Experimental conditions and study design. A study design that includes a wide range of concentrations and PK profiles provides for a good characterization of the PKPD relationship and thereby a PKPD model that can be used for reliable model predictions. A growth-control experiment in which no antibiotics are added is generally included as well as a high drug concentration when a near maximum drug effect is observed. A range of different half-lives and repeated dosing protocols might be useful for identifying time dependencies in the system. Often a single antibacterial agent’s activity against a single bacterial species is investigated at a time. However, studies of several agents (as monotherapy) against a single bacterial species could be used to support separate bacteria- and drug-specific parameters in the estimation (Nielsen et al., 2007). Similarly, a single agent’s activity against several bacterial strains might be investigated to assess the robustness of the results (Bulitta et al., 2010). Often the studies are performed with a medium that provides optimal growth conditions, but less optimal growth conditions have also been investigated, e.g., intestinal growth conditions (Andraud et al., 2011). The degree of bacterial killing by antibacterial agents is well known to be affected by the size of the bacterial inoculum, with a reduced killing rate observed against a dense bacterial population, commonly known as the inoculum effect (Udekwu et al., 2009). To characterize this phenomenon, the drug needs to be administered at both high and low bacterial inocula.

b. Combination therapy. Because of the increased prevalence of multidrug resistance, drug combinations are increasingly being used clinically to increase the therapeutic potential of available antibiotics. However, studying the time kill of drug combinations can be very tedious, as a range of doses, dosing intervals, and sequences of drug administration is being investigated. Mathematical PKPD models based on the antibiotics as single drugs can, however, be valuable for selecting study conditions that appear to be of most interest. When drug interactions exist (i.e., when the effect of the combination is smaller or larger than expected based on information on the single drugs), dynamic systems are most informative. During evaluation of drug combinations in the in vitro dynamic systems, differences in the PK properties of the two drugs should be considered. Blaser (1985) described an approach that allows simultaneous simulation of first-order elimination kinetics of two drugs with different half-lives. The elimination rate was here set according to the drug with shortest half-life, and to replace the second drug, which was eliminated too quickly, the drug was added continuously during the experiment (Blaser, 1985; Liu et al., 2004).

c. Quantification of bacterial count. For bacterial quantification, each sample is diluted in series and spread onto a series of agar plates either manually or by the use of a spiral plater. The plates are incubated for up to 24 hours, after which the number of colonies is counted manually or by the use of an automated colony counter. The lower limit of detection is generally reported to be in the range of 10–400 CFU/ml (Nielsen
et al., 2007; Bergen et al., 2010; Bhagunde et al., 2010). For experiments in which the bacteria are exposed to a high drug concentration, there has been a concern that the drug content in the sample might influence the quantification of the bacteria. One approach to minimize this so-called drug carryover effect is to centrifuge the sample and then reconstitute with sterile saline to the original volume. Subpopulations of less sensitive bacteria, showing irreversible resistance, could be quantified by culturing onto agar plates supplemented with the antibiotic in question at a concentration of 2–16 times MIC (Bulitta et al., 2010; Bowker et al., 2012; Drusano et al., 2012). To confirm the emergence of resistance, the MIC values can be established for a subset of colonies growing on the drug-supplemented agar.

d. Quantification of drug concentration. Regardless of the in vitro systems used, the PK profile simulated for the investigated drug should be confirmed by quantifying the drug concentration using an appropriate, validated analytical method. The stability of the drug can be tested by sampling directly in the time-kill curve experiments or in separate stability experiments. If the stability is assessed in separate experiments, the conditions in those experiments should mimic the ones in the original experiments as closely as possible. Use of a PK model, based on the observed drug concentration rather than the ones experimentally intended, provides for a more accurate drug exposure characterization.

B. Pharmacokinetic-Pharmacodynamic Models Based on In Vitro Data

As outlined above (section IV.A), the simplicity and flexibility of the in vitro experiments generally provide data that can support a more detailed characterization of bacterial growth and killing kinetics than data from in vivo studies. This section covers data-driven PKPD models that characterize the time course of the growth and killing in an in vitro bacterial population exposed to antibacterial drugs. Models that are not data-driven or models relating drug exposure to summary outcomes are not considered.

Data from in vitro studies have been used to support models with varying complexity, although they share the same common basic components. All models have the following: 1) a submodel characterizing the growth and natural killing of the bacteria versus time (bacterial submodel), 2) a submodel characterizing the drug concentration versus time (PK model), and 3) the interaction between these two submodels forming the full model (PKPD model). In the first section, models that do not describe any type of resistance are presented, and different ways of including the drug effect and their assumptions are illustrated. In the following section, different model structures and modeling strategies for various types of resistance are demonstrated, e.g., incorporation of heterogeneous populations with different drug susceptibilities, presence of dormant bacteria, temporary resistance in response to therapy, and the emergence of new mutants during antibiotic exposure.

1. Bacterial Submodel

The simplest mechanism-based bacterial submodel is adapted from a model developed for anticancer agents (Jusko, 1971) and involves a single bacterial compartment \( B \) with a first-order rate constant for bacterial multiplication \( k_{\text{growth}} \) and a first-order rate for the death of the bacteria \( k_{\text{death}} \).

\[
\frac{dB}{dt} = k_{\text{growth}} \times B - k_{\text{death}} \times B \tag{12}
\]

Equation 12 explains the observed exponential growth of bacteria seen in the time-kill curve experiments without the addition of drug (control experiments) as the net result of the growth rate and the rate for the natural cell death \( k_{\text{net}} = k_{\text{growth}} - k_{\text{death}} \). Often only \( k_{\text{net}} \) is estimated because of insufficient support in the data to separately define the multiplication and death rate constants. The mean generation time (MGT) or the doubling time is the average time required for the bacterial culture to double and is generally calculated based on the net growth rate according to (Garrett, 1978).

\[
MGT = \frac{\ln(2)}{k_{\text{net}}} \tag{13}
\]

In the absence of antibiotics, bacteria grow exponentially until high bacterial counts are reached and a stationary bacterial level is approached. This has been observed in the static as well as in the dynamic systems (Nielsen et al., 2011b), implying that the entry into the stationary phase is primarily attributed to reasons other than a lack of nutrition. One way of modeling this self-limiting growth is to apply a logistic function (Campion et al., 2005; Mouton and Vinks, 2005; Tam et al., 2005b) according to

\[
\frac{dB}{dt} = k_{\text{net}} \times \left(1 - \frac{B}{B_{\text{max}}} \right) \times B \tag{14}
\]

where \( B_{\text{max}} \) is the carrying capacity or the maximum bacterial count reached in the system. An alternative is to use a nonlinear function as first applied by Meagher et al. (2004) according to

\[
\frac{dB}{dt} = \frac{V_{G_{\text{max}}} \times B}{(B_{m} + B)} \tag{15}
\]

where \( V_{G_{\text{max}}} \) is the maximal velocity of bacterial growth and \( B_{m} \) is the \( B \) at which the rate of replication is half-maximal. Equation 15 was later reparameterized to be able to estimate the maximum bacterial population and the fastest growth half-life at low
bacterial densities (Harigaya et al., 2009; Bulitta et al., 2010). The self-limiting growth capacity has also been described using a model where the bacteria in the growing stage are transformed into a resting, non-growing stage when the total bacterial content in the system reaches high values, i.e., when the system is approaching the stationary phase (Nielsen et al., 2007). It is noteworthy that if several bacterial populations are defined in the model, the self-limiting growth rate should reflect the total number of bacteria and not a single subpopulation (Nielsen et al., 2007; Bulitta et al., 2010).

In some experiments, a delay in the growth of the bacteria is observed, possibly related to the fact that the bacteria are not instantaneously in their logarithmic growth phase when transferred to the culture vessel. It is common that an empirical first-order rate constant ($v$) is estimated to characterize this growth delay according to (Nolting et al., 1996).

$$\frac{dB}{dt} = k_{net} \times (1 - e^{-z\times t}) \times B \quad (16)$$

Another approach to describe a delay in the growth of the bacteria is to introduce a prebacterial compartment (Harigaya et al., 2009; Bulitta et al., 2010). In this lag compartment, the bacteria do not replicate but could, if indicated by the data, be subject to drug-induced killing. Initially (at time 0), all bacteria are stated to be in the lag compartment where they neither grow nor naturally die and where they are subsequently assumed to transfer to a proliferating bacterial compartment by an estimated first-order rate constant.

$$\frac{dB_{lag}}{dt} = -k_{lag} \times B_{lag} \quad (17)$$

$$\frac{dB}{dt} = k_{lag} \times B_{lag} + k_{net} \times B \quad (18)$$

As mentioned above, if an open dilution system is used, the loss of bacteria from the system should be accounted for in the modeling. If a dilution system with a constant flow rate is used, the correction could be done by including a first-order elimination rate ($k_a$) of the bacteria (Harigaya et al., 2009) according to eq. 19.

$$\frac{dB}{dt} = -k_a \times B \quad (19)$$

2. Pharmacokinetic Model

The PK model describes the change in the drug concentration ($C$) over time during the experiment. For the simplest system, the static time-kill curve system, the drug concentration is generally expected to be constant. However, some drugs show instability under the experimental conditions resulting in a decrease in the drug concentration over time. Therefore, to measure the drug concentration during the experiment is necessary. The degradation is often assumed to follow either a zero-order process (eq. 20) or a first-order process (eq. 21) characterized by corresponding degradation rate constants ($k_{deg}$) (Zhi et al., 1986; Nielsen et al., 2007; Schmidt et al., 2009).

$$\frac{dC}{dt} = -k_{deg} \quad (20)$$

$$\frac{dC}{dt} = -k_{deg} \times C \quad (21)$$

In the dynamic experimental systems, the drug concentration is generally simulated to decrease according to a predefined first-order elimination rate ($k_e$).

$$\frac{dC}{dt} = -k_e \times C \quad (22)$$

As described above, different experimental setups can be used to simulate more complex kinetic profiles, including an absorption profile with a first-order absorption rate constant ($k_a$) or a two-compartment disposition profile with a central and a peripheral compartment characterized by an ordinary elimination rate constant ($k_e$) as well as transfer rates between two compartments ($k_{12a}$, $k_{21}$) (Delacher et al., 2000; Katsube et al., 2008b). When the drug concentration is measured, the PK model parameters can be estimated based on the observed data, and the predicted concentrations can be used to drive the PKPD model rather than assuming the experimentally intended PK profile (Delacher et al., 2000; Campion et al., 2005; Harigaya et al., 2009).

3. Pharmacokinetic-Pharmacodynamic Model

In the full PKPD model, the bacterial submodel and the PK model are combined and equations are introduced that characterize the effect that the antibacterial drug imposes on the bacteria. The antimicrobial effect is generally assumed to be nonlinearly dependent on the drug concentration and modeled using an ordinary $E_{max}$ (sigmoidicity factor = 1) or a sigmoidal $E_{max}$ model (see section II.B). The effect could be hypothesized to either inhibit the bacterial growth rate ($k_{growth}$) as in eq. 23 (Harigaya et al., 2009) or to enhance the bacterial killing rate ($k_{death}$) as in eqs. 24–25 (Nolting et al., 1996; Mouton et al., 1997). The drug effect can be included either as a proportional effect according to eq. 24 or by an additive effect according to eq. 25.

$$\frac{dB}{dt} = k_{growth} \times \left(1 - \frac{E_{max} \times C(t)}{EC_{50} + C(t)}\right) \times B - k_{death} \times B \quad (23)$$
\[ \frac{dB}{dt} = k_{\text{growth}} \times B - k_{\text{death}} \times B + \left( \frac{E_{\text{max}} \times C(t)}{EC_{50}^\gamma + C(t)^\gamma} \right) \times B \]  
(24)

\[ \frac{dB}{dt} = k_{\text{growth}} \times B - k_{\text{death}} \times B - \left( \frac{E_{\text{max}} \times C(t)}{EC_{50}^\gamma + C(t)^\gamma} \right) \times B \]  
(25)

With time-constant parameters and no estimation of interexperimental variability, the choice of a proportional- or additive-effect implementation does not influence the goodness of fit of the model to the data; however, it is important to note that the interpretation of the parameter estimates differs between the two implementations. In eq. 24, the effect is implemented as a fractional increase in the natural bacterial death rate \( (E_{\text{max}} \times C(t)) \) (natural bacterial death rate, no unit), whereas in eq. 25, the effect is the bacterial killing-rate constant imposed by the antibiotic treatment \( (E_{\text{max}} \times C(t)) \) (second-order kill rate constant, unit hour\(^{-1}\)). Because the effect is dependent both on \( C \) and \( B \), the equations are of second order. If the data show no saturation in the effect, even at high drug concentration, the \( E_{\text{max}} \) function could be simplified as discussed in section II.B, e.g., eq. 25 could be reduced to a linear function with the only estimated parameter being a second-order kill rate constant (Zhi et al., 1986; Mager et al., 2003; Bulitta et al., 2010).

A potential time delay between the addition of drug and the antibacterial effect has been explained by an effect compartment, with the effect delay characterized by a first-order rate constant \( (k_{\text{eo}}) \) (Sheiner et al., 1979). The effect compartment is generally introduced without affecting the mass balance in the central compartment and described according to eq. 26.

\[ \frac{dC_e}{dt} = k_{\text{eo}} \times C - k_{\text{eo}} \times C_e \]  
(26)

The effect compartment model introduces a concentration-dependent effect delay, and \( C \) is substituted by the effect compartment concentration \( (C_e) \) in the concentration-effect model. Alternatively, an effect delay can be assumed to be a system property caused by depletion of a pool of cell wall constituents \( (CW) \) necessary for bacterial replication (Bulitta et al., 2009), where the drug is assumed to inhibit the production of the turnover of cell wall constituents \( (k_{\text{out},CW}) \) according to

\[ \frac{dCW}{dt} = k_{\text{out},CW} \times \left( 1 - \frac{C(t)}{IC_{50,CW} + C(t)} \right) - k_{\text{out},CW} \times CW \]  
(27)

\[ \frac{dB}{dt} = k_{\text{net}} \times \left( 1 - \frac{B}{B_{\text{max}}} \right) \times CW \times B \]  
(28)

The empirical equation described for the characterization of a delay in the growth of the bacteria (eq. 16) has also been used to characterize a time delay for the drug effect (Liu et al., 2005; Treyaprasert et al., 2007). Since the effect delay in this equation is dependent on the cumulative time after the start of the experiment, this implementation is not appropriate when modeling or simulating the antibacterial effect following repeated dosing strategies.

There are a few examples where the drug effect has been introduced in a more mechanistic fashion. Bulitta et al. (2009) have proposed a modeling strategy in which the life-cycle of the bacteria is assumed to consist of two states, represented by two bacterial compartments (Bulitta et al., 2009; Okusanya et al., 2011). The first state represents bacteria at the beginning of the life cycle, immediately after doubling, and the second state occurs immediately before doubling. The transitions between the two states were modeled as first-order rate constants. The transition between state one and state two represents the rate of bacterial replication (or MGT), i.e., the time it takes for a bacteria immediately after doubling to become ready for the next doubling. The transition between state two and one represents the doubling of the bacteria and is assumed to occur rapidly (fixed to a high value). One advantage with this modeling strategy is that it allows for flexibility, as the drug could either be set to influence the rate of bacterial replication growth (transition from state one to state two) or to influence the probability of having a successful doubling (transition from state two to one) (Okusanya et al., 2011). Another example of a more mechanistic approach to model the drug effect has been proposed for the modeling of bactericidal activity of colistin (Bulitta et al., 2010). In this model, a receptor-occupancy model was used to describe the competitive binding between colistin, Mg\(^{2+}\), and Ca\(^{2+}\) to the outer bacterial membrane. The fraction of receptors not occupied by Mg\(^{2+}\) or Ca\(^{2+}\) was used in a sigmoidal \( E_{\text{max}} \) model to calculate the effective colistin concentration at the target site, which was modeled to be related to a second-order bacterial killing process.

a. Inoculum effect. The inoculum effect refers to the commonly observed phenomenon of a reduced antibacterial drug effect observed against dense bacterial populations (Udekwu et al., 2009). Because the bacterial burden in different clinical infections is expected to show a wide variation, a PKPD model able to explain the inoculum effect might improve its predictive capacity for clinical antibacterial drug effects. The underlying mechanism of the inoculum effect is not well known. Some have suggested that it relates to a reduction in the effective drug concentration in the medium (e.g., due to denaturing enzymes or binding to viable or killed bacteria), a reduced ratio between the number of drug molecules and bacteria, or...
to differences in the physiologic state of the bacterial cells. During recent years, several models have been proposed to characterize the inoculum effect. In the model described by Nielsen et al. (2007, 2011b), normally growing bacteria are assumed to transform into a nongrowing state as a function of the total bacterial density. Because these nongrowing bacteria are expected to be drug insensitive, the consequence is a reduced drug susceptibility when the bacterial count is increasing and approaching the stationary phase. Bulitta et al. (2009, 2010) have described a model characterizing the inoculum effect by assuming that all viable bacteria synthesize and release signal molecules. These hypothetical signal molecules are expected to be related to the cell-to-cell communication associated with quorum sensing, leading to reduced drug susceptibility. The signal molecules were also modeled to inhibit the bacterial growth rate and to cause an inoculum effect by counteracting the drug effect (Bulitta et al., 2009, 2010). Furthermore, empirical modeling strategies have characterized the inoculum effect either as a bacterial density-dependent increase in the EC_{50} parameter (Udekwu et al., 2009) or as a consequence of a decreasing effective drug concentration with increasing starting inocula (Baghunde et al., 2010). One limitation of the latter model is that the decrease in killing effect is only related to the baseline inoculum. Therefore, this model lacks the ability to predict the change in drug susceptibility as a consequence of a continuously changing bacterial density.

4. Pharmacokinetic-Pharmacodynamic Models Describing Antibiotic Resistance

Several mechanisms for antibiotic resistance have been described (see section III.E). When it comes to mathematically explaining the reduced drug sensitivity in the PKPD model, both an increase in EC_{50} as well as a decrease in E_{max} for the resistant bacterial population could be hypothesized. If the mechanism of the resistance is known, this can be used as a guide in the choice of an appropriate modeling structure. If the resistance is likely to be fully overcome by a sufficiently high dose, then an increase in the EC_{50} might be used to explain the resistance. However, if the same degree of killing cannot be obtained by an increased drug concentration, then a decrease in E_{max} might be more suitable (Czock and Keller, 2007). Mathematical models can describe and provide an insight into the underlying dynamics for the emergence of resistance and thereby facilitate the design of dosing regimens that minimize resistance development.

This section will cover some of the different model structures that have been described in the literature to characterize the emergence of resistance. The choice of strategy should be based on previous knowledge of the bacterial system, the experimental design, and the observed data, e.g., by considering the mutation rate, the bacterial starting inoculum, and the indication of bacterial regrowth in the data. When using a high starting inoculum (above the natural mutation rate), there is a high probability of pre-existing resistant subpopulations being present. If a lower inoculum is used and there is no indication of regrowth in the data, a persister-type model might be expected to explain the data well. Commonly used model structures to characterize the antibacterial drug effect and emergence of resistance are presented as schematic illustrations in Fig. 5. The figure also includes model predictions of bacterial time-kill curves following either static or dynamic drug exposures. This allows the typical profiles for the different model structures to be easily compared.

a. Pre-existing resistant bacterial subpopulation.

The most frequently used modeling strategy for describing emergence of antibacterial resistance has been to assume that the total bacterial population consists of several discrete subpopulations, which differ in drug susceptibility (Mouton et al., 1997; Meagher et al., 2004) (Fig. 5C). Most often the resistant subpopulations have been modeled to be pre-existing in the starting inocula, and the fraction of bacteria in the different subpopulations at the start of the experiments is estimated as a parameter. The regrowth phenomenon is thereby attributed to a preferential killing of the susceptible subpopulation, causing a selective amplification of the less drug-susceptible subpopulation. It is common that one, two, or three subpopulations are considered in the model building process and that the size of the final model is based on goodness-of-fit criteria. The different subpopulations could be estimated to have different values of E_{max} (Bulitta et al., 2009, 2010), EC_{50} (Harigaya et al., 2009; Okusanya et al., 2011), or both (Campion et al., 2005; Tam et al., 2005a). According to the reasoning above, the mechanism for the resistance could be used to guide the selection of the affected parameter. The resistance might also be associated with a fitness cost resulting in a slower growth rate for the more resistant subpopulation (Mouton et al., 1997; Campion et al., 2005; Bulitta et al., 2009; Harigaya et al., 2009).

b. Appearance of new mutants.

A few models describe drug-susceptible bacteria mutating, becoming less sensitive, and amplifying during the experiment (Campion et al., 2005). Li et al. (1994) described a model where a growing and drug susceptible subpopulation was transformed into a growing, resistant subpopulation with a first-order mutation rate constant. In a recent publication, Wu and Derendorf (2010) proposed a model structure based on a sequential compensatory mutation hypothesis. Similarly to the former model, an initial drug-susceptible population is mutated with an estimated mutation rate into a drug-resistant nongrowing bacterial population.
is estimated to occur with the same mutation rate, whereby the fitness is restored while the drug resistance characteristics remain. Because this model structure can result in predictions similar to the model described above with pre-existing mutants, it is difficult to discriminate between the two.

c. Persistent bacteria. In some experiments, the emergence of resistance is not observed as a bacterial
regrowth but rather as lingering bacteria that seems to be protected from the killing effect of the antibacterial drug. This can be observed as a biphasic killing curve, with an initial rapid killing followed by a decline in the killing rate with time. This persistence has been modeled according to the idea of a phenotypic switching between normally growing cells and persister cells with a reduced growth rate and reduced drug sensitivity (Zhi et al., 1986; Yano et al., 1998; Nielsen et al., 2007). The transformation between these two bacterial stages is modeled by first-order rate constants. In a modification of this modeling strategy, the transformation into the resting bacterial state was assumed to be triggered by a high total bacterial level in the system (Nielsen et al., 1986; Yano et al., 1998; Nielsen et al., 2007). The transformation between normally growing cells and persister cells with an initial rapid killing followed by a decline in the killing rate with time. This persistence has been modeled according to the idea of a phenotypic switching between normally growing cells and persister cells with a reduced growth rate and reduced drug sensitivity (Zhi et al., 1986; Yano et al., 1998; Nielsen et al., 2007). The transformation between these two bacterial stages is modeled by first-order rate constants. In a modification of this modeling strategy, the transformation into the resting bacterial state was assumed to be triggered by a high total bacterial level in the system (Nielsen et al., 1986; Yano et al., 1998; Nielsen et al., 2007). The transformation was described using a linear function with the transfer rate constant (k_{SB}) equal to a proportionality constant times the total bacterial count in the system. In the parameterization of the model, the more easily comprehensible parameter B_{max}, the bacterial count in the system at stationary phase, was estimated instead of the proportionality constant. With this implementation, the two bacterial stages explain not only the biphasic killing curve but also the self-limiting growth rate.

\[ \frac{dE}{dt} = \alpha \times \left( 1 - e^{-C(t)} \right) \]

\( \alpha = 1 + \beta \times (1 - e^{-C(t)}) \times t \) (29)

The development and reversal of adaptive resistance have been modeled using a binding function with two states, adaptive resistance being off and adaptive resistance being on (Mohamed et al., 2012c) (Fig. 6A). Initially, the adaptive resistance was set to off, but as the drug concentration increased, the degree of resistance increased. The increased resistance resulted in a reduction of \( E_{\text{max}} \) and thereby a reduced capacity to kill the bacteria. When the drug diminishes, there is a reduction of the resistance and thereby a graded return of \( E_{\text{max}} \) to its initial level. To support the parameter estimation in this rather complex model structure, a wide range of in vitro experiments from static and dynamic systems with single as well as repeated dosing were included in the modeling.

5. Predictions Based on Pharmacokinetic-Pharmacodynamic Models

Once the PKPD model structure with corresponding parameter estimates have been determined, the model can be used to make predictions of untested scenarios, e.g., to support decisions on optimal dosing strategies and/or the design of future in vitro and in vivo studies. Based on model predictions, it has been shown that PKPD models developed from in vitro time-kill curve data have the ability to identify the PK/PD indices and the magnitude of these indices required for an in vivo effect (Katsube et al., 2008b; Nielsen et al., 2011a). It has also been shown that such model predictions can be used to investigate the sensitivity of the selection and magnitude of the PK/PD index toward factors such as study design and differences in PK characteristics among different patient populations (Nielsen et al., 2011a). However, even though these results verify the validity of the PKPD model, ultimately, in order to contribute to a more rational dosing regimen, the predictions should consider the full time course of the antibacterial effect. In such predictions the time course of the PK and the PD are described separately, thereby avoiding problems related the use of summary endpoints such as the PK/PD indices.

Figure 6 provides an example from our research group where a mechanism-based model describing the antibacterial effect on and development of adaptive resistance (see section IV.B.4.d) by an \( E. coli \) strain exposed to gentamicin (Mohamed et al., 2012c). The model structure (Fig. 6A) was developed based on a wide range of static as well as dynamic drug exposures, and by model simulations, the model was found to adequately describe the original data (Fig. 6B, see section VII.C). In the next step, a previously developed population-PK model of gentamicin in preterm and term neonates was used to predict the concentration-time profile following three commonly used dosing regimens for four typical newborn infants with varying gestational ages (Fig. 6C, top). The PK profiles were used to drive the PKPD model that was used to predict the antibacterial effect of the proposed dosing regimens for a certain bacterial load (Fig. 6C, bottom). Even though there is a lack of understanding of how this quantitatively translates into a clinical outcome, it allows the anticipated antibacterial effect to be visualized and different dosing regimens or drug formulations in patients with different PK characteristics to be compared (de la Pena et al., 2004). In the example above, the predictions are made without taking the...
Fig. 6. (A) Schematic illustrations of a PKPD model structure describing adaptive resistance. C, central drug compartment; P1 and P2, peripheral drug compartments (used for simulation purposes); B1, compartment with growing drug-sensitive bacteria; B2, compartment with nongrowing drug-insensitive bacteria; AROFF and ARON, compartments describing adaptive resistance being off and on, respectively; \( k_{on} \) and \( k_{off} \), rate constants for development and reversal of adaptive resistance, respectively; other parameters are described in Fig. 5. (B) Visual predictive checks (VPCs) describing the growth and killing kinetics of an *E. coli* strain after static (top) or dynamic (bottom) exposure to gentamicin. Shown are observed bacterial counts (circles), as well as median (solid line) and the 80% prediction interval (dashed line) of simulated data. The times of drug administration are indicated with arrows. The LOD (10 CFU/ml) is included as a dotted line; observed bacterial counts below LOD are plotted as 5 CFU/ml. (C) Model predictions of gentamicin concentration (top) and *E. coli* counts (bottom) for typical newborn infants with varying gestational age (GA) following three commonly used dosing regimens. Gray dashed lines represent gentamicin concentrations of 2 and 10 mg/l (top) or bacterial counts of 5 \times 10^5 (starting inoculum used in predictions) and 1 CFU/ml (bottom). Reproduced from Mohamed et al. (2012c), with permission from American Society for Microbiology.
variability or uncertainty in model parameters into account. Including variability (e.g., IV), i.e., performing stochastic simulations, would improve the value, for instance, in the determination of appropriate dosing regimens for a certain patient population.

V. Pharmacokinetic-Pharmacodynamic Modeling of Animal Data

Experimental animal models of bacterial infections have been a fundamental part of infectious disease research for more than a century and have been used to study the relationship between drug exposure and in vivo efficacy ever since the very early studies of penicillin (Eagle, 1947; Eagle et al., 1953). Today, experimental animal models are routinely used in the study of new antibacterial agents during drug discovery and development.

Even though the in vitro systems offer excellent possibilities with regard to flexibility and robustness, they can never fully represent the complex in vivo situation. In experimental animal models, the efficacy can be measured at the foci of infection, and it is also possible to study the influence of antimicrobial therapy on the pathophysiological consequences of the infection (Lutsar et al., 1997; Keel et al., 2012). Another advantage of animal models is that bacterial clearance by the immune system can be studied in its original and fully functional environment. Furthermore, even though being less flexible than in vitro systems, animal studies allow for more flexibility than usually accomplished in clinical studies, and they allow for a direct quantification of the bacterial count in the tissues (e.g., lungs or thighs).

However, it is important to recognize that the efficacy of a drug as measured in an experimental animal model will not necessarily extrapolate well to the human situation. The induction and progression of a thigh infection in a rodent will not necessarily resemble the progression of an infection in the human setting. Another limitation is that these experiments generally require a large number of animals and ethical considerations might restrict the use of larger studies with the frequent sampling necessary for characterization of the full time course of the effect. New approaches using modeling and simulation provide excellent opportunities to efficiently combine information gained by in vitro and in vivo studies and thereby use animal models in an optimal way.

A. Experimental Infections in Animals

When designing animal studies to characterize the PKPD relationship for antibiotics, several aspects need to be considered, including differences in PK characteristics between animals and humans, which have been shown to be highly influential in interpreting the PKPD characteristics of a drug (Craig et al., 1991). Another important aspect is the choice of efficacy endpoint (PD endpoint) and whether the immune system of the host should be fully functional or compromised. To improve the predictive value for human efficacy, it is generally recommended to use several different types of experimental models and to choose the route of infection with the studied bacterial species in mind.

1. Pharmacokinetics in Animals. To be able to characterize the PKPD relationship, it is essential to have knowledge about the drug exposure at the site of infection. There is generally a marked difference in the PK characteristics observed in animals (especially small animals such as mice, rats, or rabbits) and humans, with shorter half-lives in smaller animals (Gerber et al., 1986; Vogelman et al., 1988). The PK profile may also be affected by the presence of infection as well as the status of the immune system (Bedos et al., 1998; Keel et al., 2012). This could be related to the infection causing a change in the physiologic characteristics of the tissue due to the inflammatory process initiated by the host. It is preferable that the PK profile should be studied using the same experimental conditions as used for the efficacy assessments (same drug exposure, experimental infection model, immune status, etc.). Even though serum concentration is often used as a surrogate for the concentration at the actual site of infection, there are some in vivo experimental models in which the drug concentration can be fairly easy to measure at the target site, e.g., cerebrospinal fluid for the meningitis model (Lutsar et al., 1997) or the epithelial lining fluid for the pneumonia model (Rodvold et al., 2009). The microdialysis technique offers the possibility of obtaining the unbound (free) interstitial concentration at the site of infection (Liu et al., 2002; de Araujo et al., 2011). It is noteworthy that the drug concentration in tissue homogenates is generally not a good representation of the drug concentration at the target site since the drug is normally not homogenously distributed in the different compartments of tissues (interstitial fluids, intracellular fluids, different cell types, etc.).

2. Methods to Mimic Human Pharmacokinetics. Different methods have been used to more closely mimic the human PK profile in the animal experiments. One commonly applied method is to slow down the renal drug elimination in the animal by inducing a kidney dysfunction through administration of a nephrotoxic agent such as uranyl nitrate (Craig et al., 1991). Uranyl nitrate has been shown to affect both the passive filtration process (glomerular filtration rate) as well as the active kidney secretion in a dose-dependent manner in rats (Lin and Lin, 1988; Mattoes et al., 2001). As an example, administration of 10 mg/kg s.c. uranyl nitrate 3 days before the start of the experiment was shown to increase the half-life of amikacin (predominantly renally cleared) in mice from 18 to 93 minutes (Craig et al., 1991). A second alternative used to mimic the human PK in the animal is to modify the drug input. The idea is to replace the quickly
eliminated drug by administering small frequent doses or by a continuous infusion (Gerber et al., 1986; Keel et al., 2011). This method is more flexible than altering the renal elimination, and by adjusting the dose and the dosing interval or the infusion rate, the shape of the human concentration-time profile can be mimicked generally. However, the method is labor intensive, and computerized infusion pump systems allow for fairly complex drug-administration schedules (Blatter et al., 1993; Piroth et al., 1999). An alternative strategy has been to dose the animals to achieve the same PK/PD index as in humans (e.g., same fAUC/MIC or fT>MIC). This approach however has the drawback of ignoring the effect of the shape of the concentration time curve.

3. Pharmacodynamic Endpoint. In early studies, animal survival was a commonly used PD endpoint for the assessment of antibacterial efficacy (Frimodt-Møller et al., 1986; Drusano et al., 1993). Although it is a clear endpoint with a comparable endpoint in humans, disadvantages relate to the fact that it usually requires extreme bacterial burdens, which might affect the pathogenesis of the infection and the PK characteristics of the drug. Survival as a PD endpoint also provides limited information on the kinetics of the killing or the development of resistance. For these reasons, together with a better awareness of the potential stress and suffering for the animals, many investigators have substituted this endpoint with others requiring less extreme conditions. Today, the most commonly used PD endpoint is the quantification of the bacterial burden in infected tissues or fluids (Gerber et al., 1986; Knudsen et al., 1998). To quantify the bacterial burden, the animal is sacrificed, and the infected tissue is removed and homogenized in saline. Aliquots are serially diluted and plated on agar plates for CFU determination. Antibiotic-containing plates could be helpful in identifying and quantifying the emergence of bacterial populations less sensitive to the antibiotics (Gerber et al., 1982). In principle, this PD endpoint allows for a detailed PKPD characterization of the efficacy over time, although often only a single time point (e.g., at 24 hours) is evaluated. Even so, based on the bacterial burden, the common approach is to condense this information into summary PD endpoints, including the rate and duration of bacterial killing (e.g., maximal kill rate or time to reach a certain level of bacteria) or extent of bacterial killing (e.g., change in CFU at end of treatment or area metrics of the bacterial time-kill curves).

4. Immune Response. In experimental infection models the animals are often rendered neutropenic. Depending on the virulence of the pathogen and the experimental model used, this might be a necessity to allow for a successful and reproducible induction of the infection. Another reason to use neutropenic animals is that it provides a way to study the intrinsic activity of the antibiotics without the influence of the animal immune system. The efficacy as assessed in neutropenic animals would represent a worst case scenario, reflecting expected efficacy in immunocompromised patients. A common approach to induce neutropenia is to administer a total dose of 250 mg/kg cyclophosphamide divided into two intraperitoneal injections. It is common that the first dose (150 mg/kg) is scheduled 4 days before bacterial inoculation followed by the second dose (100 mg/kg) 1 day before the inoculation (Gerber et al., 1982). This regimen has been shown to produce profound neutropenia (≤10 neutrophils/mm$^3$) in outbred mice at the time of bacterial inoculation, persisting for 2 days (Zuluaga et al., 2006). Seven days after the second cyclophosphamide dose, the neutrophil count was back to normal or higher. Different cyclophosphamide dosing regimens have also been used to study the impact of various degrees of immunosuppression in the animals (Guo et al., 2011).

5. Different Animal Models. A vast number of different animal models for experimental antibacterial therapy have been described and presented in detail elsewhere (Zak and Sande, 1999). In this review, a brief description of the most commonly used models is provided. Mice and rats remain the preferred experimental animals for these studies due to low cost and ease of handling. If a poorly virulent bacterial strain is tested, a high inoculum and/or immunocompromised animals might be required in order for a successful and reproducible induction of the infection (Azoulay-Dupuis et al., 2005). An alternative when the pathogen is not naturally virulent is to use an adjuvant to inhibit the acute immunologic reaction of the animal. One of the most commonly used adjuvants is mucin, which inhibits the local macrophage function for 2–3 hours (Knudsen et al., 1995; Laohavaleeson et al., 2008). The time to initiate the antibacterial therapy is dependent on the factors affecting the time it takes to develop the infection, including the virulence of the pathogen, the inoculum size, and the status of the immune state.

a. Thigh infection model. The mouse thigh infection model was first introduced by Eagle et al. (1953) and has been further developed and widely used by others (Kunst and Mattie, 1978; Gerber et al., 1982; Hoogeterp et al., 1988; Vogelman et al., 1988; Craig et al., 1991; Jumbe et al., 2003; DeRyke et al., 2007; Andes et al., 2009; Dudhani et al., 2010; de Araujo et al., 2011). The thigh infection is produced by injecting bacteria ($10^5$–$10^6$ CFU in a volume of 0.1 ml of broth) into the thigh of anesthetized mice. It is common that the mice are rendered neutropenic by intraperitoneal injections with cyclophosphamide (see above), but immunocompetent animals can also be used to study the impact of leukocytes (Andes and Craig, 2006; Craig et al., 2010). Antibiotics are usually administered subcutaneously 1–2 hours after the thigh inoculation. For bacterial count measurement, the mice are killed, and the thigh is removed and homogenized in saline. An experimental thigh infection model has also been described for rats (de Araujo et al., 2011).
b. Pneumonia models. Several animal respiratory infection models have been described. In general, the animals are anesthetized and inoculated with approximately 10–50 µl (mice and rats) or 0.5 ml (rabbits) of a bacterial suspension (10^5–10^6 CFU) (Bakker-Woudenberg et al., 1982; Azoulay-Dupuis et al., 1999). The bacteria can be administered intranasally (Tateda et al., 1996), orally with a nasal blockage until the challenge inoculum is aspirated (Laohavaleeson et al., 2008), or by the intratracheal or intrabronchial route (Azoulay-Dupuis et al., 1991; Smith and Abbott, 1994). Diffuse pneumonia can be induced by exposing mice in a closed chamber to a bacterial aerosol generated by a nebulizer (Leggett et al., 1989). Initiation of the antibiotic therapy varies widely (from 2–3 hours up to 2–3 days after the bacterial inoculation) depending on the virulence of the pathogen and the immune status of the host. For bacterial counts, the lungs are removed and homogenized in saline. Samples for culturing can also be obtained from blood as well as pleural fluid (Bakker-Woudenberg et al., 1982).

c. Peritonitis/bacteremia models. In the peritonitis model, mice (Frimodt-Moller et al., 1986; Gerber et al., 1986) or rats (Woodnutt et al., 1992) are infected by intraperitoneal injection of organisms (10^7–10^8 CFU) in a 0.25- to 1.0-ml saline suspension that often contains mucin (Woodnutt et al., 1992; Knudsen et al., 1995). Drug treatment is initiated 1–6 hours after inoculation, depending on the time for the bacteria to reach a suitable in vivo start bacterial count. After sacrificing the animal, blood and peritoneal fluid are collected and plated for CFU determination. Growth curves in peritoneal fluid have been found to parallel growth curves in blood with an increase in variability at 24 hours postchallenge (Knudsen et al., 1998).

d. Meningitis models. Rabbits are typically used to study experimental meningitis. In this experimental infection model, immunocompetent rabbits are anesthetized and immobilized in stereotactic frames for study experimental meningitis. In this experimental meningitis model, mice (Frimodt-Moller et al., 1986; Gerber et al., 1986) or rats (Woodnutt et al., 1992) are infected by intraperitoneal injection of organisms (10^7–10^8 CFU) in a 0.25- to 1.0-ml saline suspension that often contains mucin (Woodnutt et al., 1992; Knudsen et al., 1995). Drug treatment is initiated 1–6 hours after inoculation, depending on the time for the bacteria to reach a suitable in vivo start bacterial count. After sacrificing the animal, blood and peritoneal fluid are collected and plated for CFU determination. Growth curves in peritoneal fluid have been found to parallel growth curves in blood with an increase in variability at 24 hours postchallenge (Knudsen et al., 1998).

e. Endocarditis models. To produce endocarditis, first sterile aortic vegetations are produced in anesthetized rats or rabbits by placing a sterile polyethylene catheter through the right carotid artery across the aortic valve (Archer and Fekety, 1976; Heraief et al., 1982; Wright et al., 1982). Bacterial endocarditis is induced 1–4 days later by an intravenous bacterial challenge (10^5–10^8 CFU in 0.5–1 ml of saline). Antibiotic treatment starts 1–2 days after the inoculation. At the end of the experiment, the animals are killed, and the aortic vegetations are excised, homogenized in saline, and plated for bacterial counts.

f. Skin and soft tissue infection models. Numerous skin and soft tissue infection models have been described, including models for wounds, skin abrasion, burns, and abscesses, and a variety of foreign body materials and skin traumas have been used to promote the infection (Dai et al., 2011). To produce a deep infection, the bacteria are commonly injected subcutaneously, using microbeads as abscess promoters (Bunce et al., 1992). For studying superficial infections the tape-stripping method can be applied (Kugelberg et al., 2005). Here, the fur and most of the epidermal layer are removed using elastic adhesive bandage, and the bacteria are added directly on the damaged skin area. It is common that hairless or shaved mice are used in these models and that the efficacy is evaluated based on histological examination, the size of the lesions, and the bacterial burden, determined by homogenizing, diluting, and plating of the infected skin area. Methods have also been described that utilizes in vivo imaging to noninvasively and longitudinally monitor the bacterial burden (Cho et al., 2011).

B. Models of Summary Pharmacodynamic Endpoints

The relationship between drug exposure and effect using data from experimental animal studies is commonly characterized using summary PD variables. For instance, the bacterial count after 24 hours of drug exposure is often related to the 24-hour total dose of the antibacterial drug using a sigmoidal E_{max} model (see section II.B) (Craig et al., 1991). This strategy has the disadvantage of not being able to reflect the time-related aspects of the PK or the PD of the drug. Another strategy has been to explore the correlation between the summary PD endpoint and the three PK/PD indices as follows: 1) fC_{max}/MIC, 2) AUC/MIC, and 3) fT > MIC (see section III.B) (Craig et al., 1991). A vast number of animal studies have been conducted with the aim of identifying which of these PK/PD indices best predicts the effect of various antibiotic and pathogen combinations and to determine the magnitudes of the PK/PD index required for efficacy (and/or preventing resistance).

The most commonly used PD endpoint is the measured bacterial count after 24 hours of treatment, often expressed as the change in log CFU in the tissue compared with untreated animals (Vogelman et al., 1988; Andes et al., 2009). Other PD endpoints include the mortality after 3–4 days of therapy (Drusano et al., 1993) or the maximum bacterial kill rate (Lutsar et al., 1998). Most often the serum concentrations are used to assess the PK/PD index. More seldom, the concentration at the target site, such as the concentration in cerebrospinal fluid (Lutsar et al., 1998) or epithelial lining fluid (Rodvold et al., 2009), is used.

As indicated in section III.B, the three PK/PD indices are usually highly correlated, and to increase the possibility of discriminating between the indices, a dose-fractionation
study design is needed (Vogelman et al., 1988). In these studies, the same total daily dose is divided into smaller doses and administered at different dosing intervals. Dividing the dose into smaller doses will decrease the peak concentration while maintaining the same daily AUC. For $T_{\text{MIC}}$, the result of the dose fractionation is less intuitive, being dependent on the PK characteristics of the drug (single vs. multicompartmental) and the achieved drug concentration in relation to the MIC.

In general, the drugs with shortest half-lives are the drugs for which the $fT_{\text{MIC}}$ is the major predictor of the effect. For example, $fT_{\text{MIC}}$ was the PK/PD index that was most predictive of the effect of amikacin in neutropenic mice with normal renal function (halflife of 20 minutes); however, when the half-life was increased to 2 hours, the AUC/MIC became the most important PK/PD index (Craig et al., 1991). Similarly, the choice of dosing regimen of the drug in the study might influence the conclusions. For the macrolides, the antibacterial activity was initially regarded as being best related to the $fT_{\text{MIC}}$ when studied in mice, but when the data with the largest fluctuations, i.e., data from once or twice daily dosing, were excluded, the AUC/MIC was the selected PK/PD index (Craig et al., 1991).

For fluoroquinolones and β-lactams, animal studies have shown that the magnitude of the PK/PD index is reasonably similar for drugs within the same drug class, across different bacterial strains and different sites of infection (thigh and lung), as long as the unbound drug levels are used in the assessment of drug exposure (Andes and Craig, 2002). However, it has also been reported that carbapenems require a shorter $fT_{\text{MIC}}$ than penicillins, which in turn require a shorter $fT_{\text{MIC}}$ than the cephalosporins and that the AUC/MIC required for good efficacy with fluoroquinolones might be lower for Gram-positive pathogens than for Gram-negative pathogens (Andes and Craig, 2002). There have also been studies showing a good correlation between the bacterial count at 24 hours and the survival at day 5 (Andes and Craig, 2002), although there was no strong correlation between mortality at 7–12 days after end of fluoroquinolone therapy and AUC/MIC. This was speculated to be the consequence of regrowth of persistent bacteria, implying that total bacterial eradication is not only dependent on the total drug exposure but also on other factors such as the shape of the concentration-time profile. For other drug classes, the studies are more scarce, making it difficult to draw conclusions regarding the extrapolation properties of the PK/PD index.

The PK/PD index is generally determined based on studies in which the host’s immune system has been compromised. Several studies have shown that the magnitude of the PK/PD index required to achieve a certain antibacterial effect (either measured as reduced bacterial density or survival) is lower in animals with a functional immune system, with approximately 2- to 6-fold higher doses needed for neutropenic animals (Mattoes et al., 2001; Andes and Craig, 2007; Craig and Andes, 2008). As an example, for telithromycin, a macrolide antibiotic, the magnitude of the AUC/MIC value required for efficacy in respiratory tract infections was reported to be lower in humans than in animals (Ambrose et al., 2007). It was speculated that one main reason for this discordance was that the animals were rendered neutropenic, and the authors state that the AUC/MIC value for efficacy would decrease by >3- to 4-fold in immunocompetent mice, further highlighting the sensitivity in the PK/PD index value for experimental conditions.

As discussed above (see section III.B), the use of the PK/PD indices have several drawbacks. As the efficacy endpoint in the animal studies most often is restricted to a single 24-hour observation time point, much information on the time dependence of bacterial growth and killing is lost. In addition, 24 hours is generally a relatively short period to study the adaptation of the bacteria to antibiotic drug exposure and selection of resistant bacterial subpopulations. Therefore, the PK/PD indices ignore essential parts needed to achieve an optimal antibacterial dosing regimen.

C. Models Characterizing Full Pharmacodynamic Time Courses

Examples of PKPD models characterizing the full time course of antibacterial effects based on animal data are still relatively scarce. The aim of such studies has generally been to provide an in vivo evaluation of the predictive performance of model structures previously developed based on rich in vitro data.

1. Pharmacokinetic-Pharmacodynamic Models Without Antibiotic Resistance. For in vitro data, the simplest and most commonly applied mechanism-based PKPD model for antibacterial agents includes a first-order growth rate constant describing the exponential net growth rate of the bacteria and an additive antibacterial killing effect (eq. 25; section IV.B).

This model structure has also been shown to be applicable for characterizing the growth and killing kinetics following antibacterial drug treatment in vivo (Zhi et al., 1988; de Araujo et al., 2011). In 1988, Zhi and colleagues used the murine peritonitis model to follow the bacterial count data over time (every 10 minutes during 3 hours) after different drug exposures (Zhi et al., 1988). As PD endpoint the authors used the ratio of the observed bacterial count measurements in whole blood to the bacterial count in the start inoculum. Estimated PKPD parameters included the net growth rate constant ($k_{\text{net}}$), $E_{\text{max}}$, and EC$_{50}$. The same model structure was applied by de Araujo et al. (2011) to in vivo data obtained using the thigh infection model in immunocompromised rats, and the bacterial counts were assessed every 2–3 hours during 24 hours. In this study, the experimental setup was the same as in a previously performed in vitro time-kill study (same bacterial strain and antibiotic) (Nolting et al., 1996),
which allowed the authors to compare the parameter estimates between in vitro and in vivo conditions. The free drug concentration at the infection site, determined by microdialysis, was used in the modeling. The growth rate constant obtained in vivo was lower than what was observed in vitro (0.75 vs. 1.30 hour\(^{-1}\)), which is in accordance to several other studies (Johanson et al., 1974; Katsube et al., 2008a). Furthermore, the estimated values of the drug-related parameters (\(E_{\text{max}}\) and \(EC_{50}\)) were lower in vivo than in vitro. However, these results should be interpreted with some caution since the data from the different dosing regimens were not modeled simultaneously, limiting the available information content regarding the full PKPD relationship.

2. Pharmacokinetic-Pharmacodynamic Models Describing Antibiotic Resistance. More complex PKPD model structures have been verified to be applicable when characterizing the full time course of bacterial killing observed in vivo. Jumbe et al. (2003) applied a PKPD model with multiple pre-existing bacterial subpopulations, differing in drug susceptibility, to data obtained in a mouse thigh infection study with bacterial count measurements (six occasions during 24 hours) following single drug doses. Homogenates of the thigh were cultured on drug-free agar plates (total bacterial count) and onto drug-containing plates (drug-resistant bacterial count) for quantification of the bacterial count. The total bacterial population was assumed to consist of two discrete pre-existing subpopulations. The PKPD model structure for each subpopulation was similar to the one used by Zhi et al. (1988) and “Yano et al. (1998) (see section IV.B) with the addition of a self-limitation in growth (logistic growth model) and a sigmoidicity factor in the \(E_{\text{max}}\) model. The two subpopulations were assumed to have different values for the first-order growth rate constant and different drug sensitivity as reflected by different estimates of the \(E_{\text{max}}\) parameter.

Full time-course animal data have also been used to evaluate the PKPD model structure originally presented by Yano et al., (1998) (see section IV.B). This model consists of two bacterial compartments, here representing growing drug-sensitive bacteria and resting drug-insensitive bacteria. The model was applied to bacterial count data (eight occasions during 12 hours) obtained from the lung infection model in immunocompromised mice (Katsube et al., 2008a). Because of the limited amount of information available in the in vivo data, the parameters describing the transfer rates between the two bacterial compartments were assumed to be the same for the in vitro and in vivo experiments and consequently not re-estimated for the in vivo data. Furthermore, it was assumed that the \(EC_{50}\) could be calculated based on the MIC value according to a relationship between the \(EC_{50}\) and MIC previously obtained from in vitro time-kill experiments for drugs belonging to the same class of antibiotics. With these assumptions, only the growth rate constant and the \(E_{\text{max}}\) were re-estimated based on the in vivo data. By use of the parameters from the in vitro experiments, the proposed PKPD model could describe well the in vivo bacterial count in animal-infection model experiments.

3. Models Including the Immune System. The animal models also provide an opportunity to study the time course of the antibacterial effect imposed by the host’s immune system. Some recently published articles have used mathematical models to quantitatively describe the impact of immune cells on bacterial clearance (Drusano et al., 2010a, 2011a,b; Guo et al., 2011).

One approach has been to use different baseline bacterial burdens to study the impact of the granulocytes on the bacterial killing (Drusano et al., 2010a, 2011a,b). These studies have been performed using both the thigh and pneumonia mice models with a noncompromised (normal) immune system. The authors concluded that the ability of the granulocytes to kill bacteria was saturable at high bacterial burdens, as the immune cells alone reduced the bacterial burden over time when using low start inoculum, whereas at higher initial burdens (greater than approximately \(10^7\) CFU per gram of tissue), a net bacterial growth was observed over the studied 24-hour period. The immune system was estimated to be saturated by half at an approximate bacterial burden of \(2\times10^8\) CFU/g. The rate of change in bacteria was expressed as the difference between the growth rate of the bacteria and the kill rate imposed by the neutrophils and modeled according to eq. 30. The same model structure for bacterial killing of granulocytes has also been used to describe the combined effect of granulocytes and drug on bacterial cell kill (Drusano et al., 2011a).

\[
\frac{dB}{dt} = k_{\text{growth}} \left(1 - \frac{B}{B_{\text{max}}} \right) \times B - \frac{E_{\text{max}} \times B}{B_{50} + B} \times B \quad (30)
\]

Another approach to quantitatively describe the impact of the immune cells on bacterial clearance has been to follow the bacterial burden in mice with various degrees of immunosuppression (Guo et al., 2011). A pneumonia model with immunocompetent mice as well as mice rendered neutropenic using three different doses of cyclophosphamide (reducing the neutrophil count approximately by 20, 70, and 90%) was applied, and the bacterial burden was followed over time (five occasions during 24 hours). A similar modeling approach was used as described above but here the kill rate imposed by the neutrophils was saturable with respect to the absolute neutrophil count (ANC) according to eq. 31. A net bacterial growth was observed in animals with the most pronounced
neutropenia, while net bacterial killing was seen in animals with lower immunosuppression and in animals without immunosuppression. The numbers of neutrophils necessary for 50% of maximal killing (ANC\textsubscript{50}) was estimated to be 191/\muL.

$$\frac{dB}{dt} = k_{\text{growth}} \left(1 - \frac{B}{B_{\text{max}}} \right) \times B - \left(\frac{E_{\text{max}} \times \text{ANC}}{\text{ANC}_{\text{50}} + \text{ANC}}\right) \times B$$

(31)

A limitation of the studies referred to above is that the number of granulocytes was not measured over time during the experiments.

VI. Clinical Pharmacokinetics-Pharmacodynamics

The optimal clinical dose depends on the patient PK, the antibacterial effect on the infecting bacteria, potential dose-limiting side effects, and the immune status of the patient. Today, the risk of resistance development is generally not taken into account in the dose optimization despite the alarming situation of accelerating resistance development. To preserve the activity of the antibiotics, there is an urgent need to revise current methods for dose selection. PK can show pronounced variability from patient to patient, and especially in the case of severe infections, also within a patient. Variability in PK combined with differences in drug susceptibility between pathogens, site of infection, and immune status can result in large variability between patients in the PKPD relationship and outcome. Thus, there is a frequent need to adapt the dose and/or dosing schedule to the population being treated, for which PK and PKPD modeling serves as excellent tools. Although population-PK models in patients have been established for many antibiotics, there is a lack of predictive PKPD models based on clinical data. One challenge of clinical studies, and thereby for establishment of PKPD models, is the difficulty for measuring bacterial growth and kill in patients and the lack of reliable biomarkers to follow the change in disease over time. Another challenge is that patients receiving antibiotics are often critically ill and may be difficult to recruit for trials with extended sampling procedures. Another challenge is that the PKPD relationships for clinical cure and resistance development may not overlap. This section will give an overview of PK in patients and the characteristics of available models based on data from patients with bacterial infections.

A. Pharmacokinetics in Bacterial Infections

1. Pharmacokinetics in Patients. PK is typically studied in healthy volunteers in the initial drug development. The PK parameters obtained, however, often do not represent well the population to be treated with the antibiotic. When there is systemic response in patients with infections, and especially in sepsis and septic shock, the PK can be affected pronouncedly (De Paepe et al., 2002). As an example, for drugs that are cleared by the kidneys, renal failure can cause accumulation of parent drug and metabolites. Altered tissue distribution can occur due to fluid shifts, protein binding, and pH changes, but also organ perfusion and tissue binding can change as a consequence of infections (Gonzalez et al., 2011). The disease condition, in addition to drug properties such as pK\textsubscript{a} and lipophilicity governs to what degree PK is affected. For example, fluid shifts cause hydrophilic drugs to distribute to sites with edema and ascites, leading to an increase in V\textsubscript{d} and a decrease of C\textsubscript{max} in plasma. Drugs with low fraction unbound (f\textsubscript{u}) are most sensitive to changes in the protein binding. However, in theory, the unbound concentrations are affected only for high extraction drugs administered intravenously, for which elimination (CL) is independent of f\textsubscript{u} (Rowland and Tozer, 2011). The impact of protein binding for antibiotics in vitro and in vivo has recently been extensively reviewed (Schmidt et al., 2010; Zeitlinger et al., 2011). Changes in PK during the time course of infection will consequently affect the antibacterial effect and thus are important to consider in the evaluation of PKPD relationships.

Population-PK modeling is part of modern clinical drug development in this therapeutic area, and there are numerous population-PK models presented in the literature for antibiotics. For drugs primarily eliminated by the kidneys, CL\textsubscript{CR} is often found to be a significant covariate to explain parts of the variability between patients, and CL\textsubscript{CR} can thereby guide individualization of doses. Body size, often expressed as body weight, is also a recurrent covariate that is most frequently applied in the development of dosing regimens in children. In small children and neonates, maturation of organ function is also of importance (Anderson and Holfd, 2008).

For older antibiotics, PK is being re-evaluated to characterize variability and to investigate covariate relationships, and together with available information on effect and side effects, this leads to optimization of dosing schedules. Colistin is one example of an old drug that has lately gained interest for use as a “last-line” therapy and where development of a sensitive and specific assay (Jansson et al., 2009) has allowed population PK to be determined in the target patient population (Plachouras et al., 2009; Garonzik et al., 2011; Mohamed et al., 2012b). As a consequence of the findings from the PK model, a new dosing regimen was suggested with a 9-million-unit (MU) loading dose and an extended dosing interval (4.5 MU Q12h vs. 2–3 MU Q8h). This dosing regimen showed good efficacy (82% clinical cure) and acceptable toxicity (18% experienced acute reversible kidney injury) in critically ill patients with infections of Gram-negative bacteria sensitive
only to colistin (Dalfino et al., 2012). Individualized dosing based on CLCR has also been proposed (Garonziki et al., 2011), although CLCR-based dosing appears to be of limited value for reducing variability between patients with mild or moderate renal impairment (Mohamed et al., 2012a). Mohamed et al. (2012b) studied the PK on two to three occasions per patient and could thereby quantify IOV of colistin. Since IOV was pronounced and of similar magnitude as IIV, the potential for a posteriori feedback adaptation (therapeutic drug monitoring, TDM) for colistin in critically ill patients may be of limited value (Mohamed et al., 2012b). In addition to ongoing studies aimed at characterizing PK of other old drugs such as polymyxin B and fosfomycin, potential alterations in PK for concomitant antibiotics in combination regimens would also interest future investigators seeking to better understand outcomes.

2. Pharmacokinetics in Target Tissue. As discussed above, most bacterial infections are localized in the interstitial fluid in a tissue rather than in the plasma. Differences in tissue and plasma concentrations due to a reduced or delayed drug distribution may result in unexpected failure (Mueller et al., 2004). As an example, time courses in the epithelial lining fluid (ELF) may have clear differences clinically from the time course in plasma, with ELF-to-plasma concentration ratios in the range from <0.1 to 3 for antimicrobial agents (Rodvold et al., 2011). Microdialysis provides a possibility to directly measure the unbound drug concentration in accessible tissues and interstitial fluid (Joukhadar et al., 2001; Mueller et al., 2004); however, the technique is labor intensive and not applicable for many clinical infection sites. Antibiotic drug distribution into tissue may be predicted based on whole-body physiologically based PK (WBPBPK) modeling that has gained interest in drug development during recent years (Rowland et al., 2011). This modeling approach can give a better prediction of the drug concentration in a certain tissue or organ compared with the traditional compartment modeling where the compartments generally have no anatomic partition. IIV and covariates can also be linked to parameters in these types of models (Huisinga et al., 2012). Recently, Bouchene et al. (2012) illustrated how a WBPBPK model can be built based on plasma- and renal-drug concentrations, with partition coefficients and blood flows derived from the literature for partition coefficients (Bjorkman et al., 2001; Berezhkovskiy, 2004) and blood flows and tissue volumes (ICRP, 2002). The model was used to provide further understanding of colistin disposition.

B. Pharmacokinetics-Pharmacodynamics of Antibacterial Drug Effects in Patients

1. Pharmacodynamic Endpoints in Clinical Studies. In clinical studies, the antibacterial effect is generally assessed as a clinical and/or microbiological response (European Medicines Agency, 2012). Clinical response is commonly evaluated by comparing the signs and symptoms of infection before and after therapy, with clinical cure and failure defined as resolution, and persistence or worsening, respectively. Usually, this results in one dichotomous data point per patient and since each patient in the study contributes limited information to the evaluation of treatment efficacy, large trial sizes are often needed. The microbiological response can only be evaluated if it is possible to identify the infecting bacteria and preferably the MIC also should be determined. Microbiological response can be classified into one of four categories as follows: eradication, persistence (pretreatment pathogen present in post-treatment culture), superinfection (emergence of a new bacteria during therapy with worsening of symptoms), or indeterminate. Often, however, only two categories, eradication and persistence, are applied for success and failure, respectively. Potential connections between clinical and microbiological response are generally neglected, although the correlation between the two could provide additional information.

Other examples of variables that have been used to determine antibacterial efficacy include time to bacterial eradication, time to body-temperature resolution, and time to leukocyte count resolution, see e.g., Kashuba et al. (1999). Potential biomarkers such as body temperature, leukocytes, procalcitonin, tumor necrosis factor-α, interleukin-6, and C-reactive protein should be further investigated for use as predictors in a PKPD model of outcomes. For example, procalcitonin has shown promise as a guide for when to stop treatment; in addition to reducing the risk of side effects, calcitonin also reduces antibiotic consumption (Schuetz et al., 2012).

2. Pharmacokinetic-Pharmacodynamic Analysis of Efficacy. Nowadays, PK/PD indices are frequently correlated with response outcomes in the analysis of clinical trials, i.e., PKPD-PD relationships are explored. Classification and regression tree (CART) analysis is commonly applied to find the optimal breakpoint for success versus failure in the distribution of PK/PD index values (Highet et al., 1999). The CART technique identifies the cut-off value of the independent variable (e.g., the magnitude of the PK/PD index or therapy duration) where values above are related to a significantly higher chance of a positive outcome than values below. There are few failures, however, and this limits the possibility of setting cut-offs.

Compared with CART analysis, logistic regression provides a more informative type of analysis as multiple variables can be evaluated for a categorical outcome (that can be dichotomous or polytomous). In addition, the relationships can take on different shapes. Covariates, IIV (if more than one observation per individual),
and time functions can be added to the logit function described in eqs. 8–9. In addition, different shapes of the relationships can be investigated, e.g., the linear relationship to concentration on the logit-scale in eqs. 8–9 can be substituted by an $E_{\text{max}}$ model. Analyses of clinical data may also include time to eradication or relief of symptoms using Cox proportional hazard models with exposure included as a predictor. However, a parametric time-to-event analysis would be more informative, as such models are more useful for predictions and simulations.

In 1993, there was a first report on a link between the magnitude of a PK/PD index and the outcome of therapy (Forrest et al., 1993). In a multivariate logistic regression analysis, the most predictive factor for both clinical and microbiological cure for ciprofloxacin was found to be AUC/MIC. In addition, the AUC/MIC ratio was predictive of the median time for eradication. Since then, there have been several clinical studies on the effectiveness of fluoroquinolones, where AUC/MIC or $C_{\text{max}}$/MIC have been shown to be the PK/PD index with the best correlation to clinical and microbiological outcomes (see e.g., Preston et al., 1998; Ambrose et al., 2001). However, the magnitude of the PK/PD index required for effect may have been dependent on indication and infecting bacteria (Ambrose et al., 2007).

Evaluation of PK/PD indices for clinical and/or microbiological outcome in a logistic regression model has become standard practice in the analysis of clinical trials. See for example the analysis of linezolid (Rayner et al., 2003), aminoglycosides (Kashuba et al., 1999), vancomycin (Moise-Broder et al., 2004), and tigecycline (Bhavnani et al., 2012). The PK/PD indices were tested as predictors in the models together with other variables such as bacterial species and infection site. As discussed above, the PK/PD indices have several limitations that also make them less reliable as predictors for trial outcomes. Using MIC and the summary exposure as independent values may be a better approach, although using the time course of drug effect or of biomarker(s) would be our preferred modeling strategy. It would be even more informative if a (mechanistic) PKPD model describing repeated measures of bacterial counts could be built and used as the driver for probability of cure. From models characterizing the change of disease over time, treatment duration may be optimized also, without the needed for evaluating a wide range of possible scenarios in clinical trials.

3. Pharmacokinetic-Pharmacodynamic Analysis of Resistance. There are also examples where resistance has been investigated by CART analysis to identify a PK/PD index at a magnitude related to the risk of resistance (Thomas et al., 1998). A PK/PD index may, however, not be the ideal variable to correlate to resistance development. As an alternative, it has been suggested that the time spent in the mutant selection window during 24 hours ($T_{\text{MSW}}$), i.e., above MIC and below the mutant prevention concentration (MPC), is related to the development of resistance (Zhao and Drlica, 2001). In a recent simulation study, both the $\beta$AUC/MIC for effectiveness against the wild-type bacteria and the $T_{\text{MSW}}$ were integrated when evaluating the outcome of dosing regimens of ciprofloxacin (Khachman et al., 2011). On the basis of the results, the authors suggest the use of ciprofloxacin in combination with other drugs or switching the drug for patients infected with Pseudomonas aeruginosa or Acinetobacter baumannii to avoid selection of resistant bacteria. As with the killing of sensitive bacteria, models that can characterize a PKPD relationship over time that prevents selection of resistant bacteria would provide valuable information for optimizing dosing regimens.

4. Pharmacokinetic-Pharmacodynamic Analysis of Side Effects. There are few examples of models for side effects of antibiotics in the literature, even though such models could be very useful in the development of optimal dosing regimens by balancing toxicity and efficacy. In this section, two examples of longitudinal, mechanism-based models will be illustrated: one for reversible nephrotoxicity (Rougier et al., 2003) and one for thrombocytopenia (Sasaki et al., 2011). Both variables have also been modeled as categorical variables (Rybak et al., 1999; Forrest et al., 1993), but summarizing the underlying continuous data to such endpoints ignores the quantitative information on the time course and is thereby of limited use for predicting untested dosing schedules. Since both models presented below have a mechanistic basis and may be of use also for other antibiotics showing the same side effects, they are described in relative detail.

a. Reversible nephrotoxicity. Nephrotoxicity is a frequent side effect of many antibiotics. For aminoglycosides, reversible nephrotoxicity has been modeled as a saturable renal drug uptake, with the maximum rate determined by $V_{\text{max}}(t)$, leading to drug accumulation in the renal cortex (Rougier et al., 2003). The change of drug in plasma ($A_{c(\text{AG})}$) with time ($t$) was determined by nonrenal elimination described by $k_{\text{nr}}$ and renal elimination dependent on $CL_{\text{CR}}(t)$ and the tubular fraction reabsorbed ($f_{\text{reabs}}$). For simplicity, we have reduced their two-compartment model for drug distribution to a one-compartment model in the equations shown in eq. 32.

$$\frac{dA_{c(\text{AG})}}{dt} = -\left[k_{\text{nr}} + k_r \times (1 - f_{\text{reabs}}) \times CL_{\text{CR}}(t)\right] \times A_{c(\text{AG})}$$

(32)

A Michaelis-Menten function described the saturable uptake where $K_M$ is the $A_{c(\text{AG})}$ for which half of $V_{\text{max}}(t)$ is obtained. The turnover of drug in the renal compartment ($A_{r(\text{AG})}$) was governed by a saturable uptake and
First-order elimination characterized by rate constant, $k_{rc}$.

$$\frac{dA_{AG}}{dt} = -k_{rc} \times A_{AG} + \frac{V_{max}(t) \times A_{AG}(t)}{K_M + A_{AG}(t)} \quad (33)$$

Furthermore, a decreased accumulation over time [reduced $V_{max(t)}$] was explained as regeneration of tubular cells. The change in CLCR was determined by 1) a sinus function characterizing the diurnal variation in glomerular filtration rate and 2) a sigmoid $E_{max}$ model explaining the reduction in CLCR from its baseline value (CLCR(0)) caused by the drug (eq. 34).

$$\frac{dA_{AG}}{dt} = -k_{rc} \times A_{AG} + \frac{V_{max}(t) \times A_{AG}(t)}{K_M + A_{AG}(t)} \quad (33)$$

VII. Some Practical Aspects of Pharmacokinetic-Pharmacodynamic Modeling

A. Mechanism-Based Versus Empirical Models

PKPD models are useful in various different situations as follows: 1) to describe the data at hand; 2) to advance the understanding of the complexity in the interaction of the drug and the biologic system; 3) to characterize the sources of variability between patients and experiments; 4) to predict outcomes of untested situations, e.g., for dose optimization; and 5) to design new studies. Although a complex model may appear to be most appropriate, we believe that the level of detail of the model should depend on its intended purpose. To quote Box's famous statement "essentially, all models are wrong, some are useful" (Box and Draper, 1986).

Mechanism-based models are depicted as being a representation of the important underlying biologic processes and the drug's mechanism of action. Mechanism-based models are more reliable for extrapolations beyond the study conditions of the data used to develop the model, compared with more empirical models (Marshall et al., 2006; Danhof et al., 2008). However, mechanism-based models generally require considerably more developmental time than empirical models, and to quickly make a decision, an empirical model may provide sufficient insight into the degree of drug effect. The available data limits the possibility to build complex models, and although parameters may be fixed to literature values, it should be acknowledged that bias could be introduced if the fixed parameters are not fully accurate for the study conditions. The value of the model may therefore in many situations increase if it is simpler in its structure, and we suggest that the most parsimonious model should be striven for when using a data-driven approach.

In the development of a mechanism-based model, the processes that are time limiting should be prioritized for incorporation into the model structure. Parameters that have a mechanistic meaning may be included as priors, i.e., not as fixed values but as an expected values with their uncertainties (Ambrose et al., 2012). However, the modeler needs
to be careful in selection of priors to make sure the prior is representative for the population at hand.

A mechanism-based model that has been shown to be very useful is a model for chemotherapy-induced myelosuppression (Friberg et al., 2002). A reason for its usefulness is that it separates system-related parameters from drug-related parameters and only four structural parameters need to be estimated: three parameters that are system related and one parameter that is drug related. The model has found application in many steps in drug development, for example, for bridging myelosuppression in rats to humans (Friberg et al., 2010) and for describing the effect of drug combinations and modifying agents (Sandstrom et al., 2006). In addition, it has been shown to be useful for describing side effects in other disease areas (Gupta et al., 2010; Sasaki et al., 2011). Therefore, the efforts taken to develop this type of system or platform model is rewarding when it can be applied to many different projects, and extensions can be naturally added to adapt to new study conditions. As only limited information on the drug effect may be needed when the underlying system is characterized, mechanism-based PKPD models can be useful for selecting between candidate drugs.

B. Software and Technical Aspects

The development of population PK and PKPD models requires software that is based on nonlinear mixed-effects modeling where different types of variability can be estimated, e.g., IIV and IOV can be separated from residual variability. The most commonly applied software for nonlinear mixed-effects modeling is NONMEM (Beal et al., 2009), and therefore, this section is focused on applicable features in this software. Other popular software in the infectious diseases area include ADAPT based on an importance-sampling algorithm (Bauer, 2008; D’Aregnio et al., 2009), MONOLIX based on Markov Chain Monte Carlo (MCMC) Stochastic Approximation Expectation Maximization (SAEM) (Lavielle, 2012), and the Non-Parametric Adaptive Grid (NPAG) program (Leary et al., 2001). Up until version VI, the first-order (FO) and first-order conditional estimation (FOCE) were the algorithms of choice in NONMEM. In the version 7 release, it is also possible to use SAEM, importance sampling expectation maximization (IMP) methods, and an MCMC method (BAYES). The FOCE and sampling methods appear to be faster than the MCMC methods in general, although computational times can be much influenced by changing convergence criteria settings. Reduced run times in practice enable the evaluation of more models, with more sophisticated model structures and variability distributions. Bias in model parameters has in general been shown to be low for all methods, whereas FOCE is typically the most robust (Johansson et al., 2010; Gibiansky et al., 2012; Plan et al., 2012). S-ADAPT and FOCE in NONMEM have been shown to produce similar parameter estimates for PKPD analysis of time-kill curve data (Bulitta et al., 2009). Software programs are continuously being updated to improve on speed, robustness, and user friendliness, as well as the addition of new features.

NONMEM is one software program that allows adequate handling of variability in data from in vitro time-kill curves. Ultimately, all available bacterial counts at any time point should be included in the analysis, as analyzing averages of measurements can introduce bias. For replicate observations, i.e., more than one observation per experiment and sampling time point, a shared residual error can be applied using the level 2 (L2) data item in NONMEM, i.e., the same idea that has been applied when simultaneously analyzing parent and metabolite data. The variability between bacteria samples drawn at the same time point and from the same tube is likely to be lower than the variability between other samples because they are dependent on exactly the same start inocula, exactly the same drug concentration, and the same potential deviation in time of sampling. Ignoring correlations between replicate samples might introduce bias (Karlsson et al., 1995), and in our experience, a shared residual error has been of statistical significance and has stabilized the model fitting. Intereexperimental errors can be included in the model by specifying one experiment as one individual (ID), for example, as described in eq. 10. However, our experience is that allowing for intereperimental variability in the analysis of time-kill experiments often cause model instability, with overestimated variability and trends of bias in the parameters of primary interest in this type of analysis, i.e., the typical parameter estimates. The estimation of autocorrelation between observations can also be applied to account for the fact that samples drawn from the same experiment are correlated. Data from in vitro time-kill curve experiments typically cover a very wide data range from $10^1$ to $10^9$ CFU/ml. Estimation based on transformed data, e.g., log-transformed, is therefore recommended. When samples are below the limit of detection (LOD), they could either be omitted if they are few, or as recommended, the M3 method could be used when the samples below LOD are treated as categorical values and handled using a likelihood-based approach, estimating the probability of observing data <LOD (Beal, 2001).

C. Model Evaluation

During model development, diagnostics to evaluate model performance should be applied to investigate any trends in predictions and residuals that may reveal model misspecification. The traditional plots of observations versus predictions are often of limited use. A correct model might appear incorrect due to the study design and as model misspecification can be
hidden due to shrinkage in variability parameters (Karlsson and Savic, 2007). For similar reasons, we find the $R^2$ value to be of limited use to evaluate if a model is a good representation of the data at hand, e.g., the $R^2$ value is less likely to be high for a design where sampling is made in the most informative and challenged parts of a biological system, whereas the $R^2$ value will show a perfect correlation for the extreme design of only two samples (Sheiner and Beal, 1981). However, the $R^2$ value may be useful for comparing model fits from experiments with the same design and is commonly used to assess the PK/PD indices.

As long as the number of experiments and individuals are not extensive, we find plots of observations and predictions of subjects or individual experiments versus time to be most informative, in addition to simulation-based diagnostics such as the visual predictive check (VPC) (Yano et al., 2001; Karlsson and Savic, 2007). In VPCs, a number of replicate datasets (e.g., 500) are simulated from the model to be evaluated, using the original dataset as a template. The median and outer percentiles (e.g., 10th and 90th) of the simulations are computed and the observed data points overlaid together with the median and percentiles of the observed data. This allows for judgment of model fit, given the degree of variability in the data. Both the structural and variability models are thereby evaluated in the VPC and by stratification the model fit for each experiment can be illustrated and further guide model development. Figure 6B shows an example of a VPC for in vitro time-kill curve experiments for *E. coli* exposed to gentamicin. The majority of the experiments are well characterized by the model as the data are scattered around the predicted time course and within the prediction intervals. Illustrating the data and model performance in a VPC also allows the reader to evaluate potential weaknesses. There are, for example, signs of underprediction at 4 and 6 hours for 1 mg/l and overprediction at 9 and 12 hours for 2 mg/l (Fig. 6). Given the complexity of the underlying processes of the modeled system, including adaptive resistance, we judged the model to describe the experiments adequately.

Parameter uncertainty is also of importance in the judgment of how reliable the model is for extrapolation and simulation. Most software provides a measure of parameter uncertainty, but techniques such as the nonparametric bootstrap is also frequently applied. In a nonparametric bootstrap, individuals (experiments) are randomly sampled, with replacement, from the original dataset to form new datasets with the same number of individuals as the original dataset. Parametric (based on standard errors) and nonparametric 95% confidence interval of the parameters is computed from the distribution of the model parameters estimated from the replicates. With the nonparametric bootstrap technique, asymmetry in the parameter distributions can be detected. For in vitro experiments, where each experiment is designed to contribute unique information, bootstrapping the experiments may lead to unbalanced information in each replicate data set and thereby result in inappropriate estimation of parameter uncertainty. An alternative method to obtain standard errors for these studies is log-likelihood profiling. In this method, the confidence intervals are obtained by finding the fixed parameter values around the estimated value that result in the 95% confidence limit.

VIII. Future Perspectives

As mentioned, there is a lack of effective antimicrobials in the drug-development pipeline, despite the critical need for new antibiotics. Especially urgent is the need to develop antibacterial agents with new mechanisms that are not sensitive to a single mutation that can confer drug resistance. To increase the efficiency and attractiveness of drug development in the antibiotic area, we believe a quantitative, model-based methodology is imperative. The computational power exists and pharmacometrics have been successfully applied in most major disease areas to rationalize drug development. However, modeling and simulation techniques have further potential, and for antibiotics, these approaches could be used more efficiently to explore different dosing schedules and trial designs and maximize the information derived from preclinical and clinical studies. The models can provide a link between preclinical (in vitro and animal) data to clinical responses. As good preclinical experimental models exist for bacterial infections, pharmacometrics provides an excellent path for translation of the full time courses. There are several examples of successful model development and implementation of model-based drug development for other infectious diseases, such as HIV and hepatitis C virus (HCV) (Rosario et al., 2005; Jacqmin et al., 2010; Adiwijaya et al., 2012).

Once models of both desired effects and side effects are available, they can be integrated to support optimal dosing regimens and go/no-go decisions. The models can be used to develop risk (penalty) functions to weigh benefits (bacterial cure) and risks (resistance development, toxicity, and excessive dosing), which has been nicely exemplified by Viberg and coworkers (2008). Model-based approaches can also be used for optimizing cut-off values for dose individualization of dosing strategies based on covariates (Jonsson and Karlsson, 2003). Model-based meta-analysis is also an emerging technique within the pharmacometrics area that can be valuable for early assessment of the effect in relation to comparators (Mould, 2012).

Clinical studies specifically aimed at infections with resistant bacteria are still likely to be relatively small in size, but in an ideal case, once the concentration-effect
relationship has been identified, only small clinical trials may be needed to confirm the outcome. It has been suggested that antibiotic drug development should be based on development strategies similar to those for orphan drugs (Bashaw et al., 2011) where quantitative approaches have a key role in decision making during all phases of clinical development. Furthermore, with model-based analysis, the number of patients needed can be reduced substantially while preserving the statistical power of the study (Vong et al., 2012). Optimal design methodology provides a further possibility for increasing the efficiency of experimental and clinical study designs. Not only time points for measurements can be optimized for informativeness (Nyberg et al., 2012) but the technique can also be applied to other design specifics, such as dose levels, number of doses, number of patients per study arm, and experimental conditions to test. This technique becomes even more important as the number of factors increases, e.g., when exploring combination therapies.

One of the limitations to application of a model-based approach, however, is the lack of skilled personnel and the need for training pharmacometricians to meet the demand (Holford and Karlsson, 2007; Barrett et al., 2008). To move forward more quickly without duplication of efforts, it is also essential to collaborate and to share data and model code. DDMoRe [http://www.ddmore.eu/; (Harnisch, 2013)] is a 33-million-Euro-budget European initiative, funded by Innovative Medicines Initiative (IMI), European Federation of Pharmaceutical Industries and Associations (EFPIA), and academia, working toward setting up a searchable library for pharmacometric models. In addition to model code and associated data, the library will include components to translate models from one coding language to another to facilitate sharing of models between pharmacometricians in industry and in academia. Up to date, pharmacometric models have primarily been applied in clinical drug development, but in the future, models may be integrated into clinical care and thereby provide a way to advance personalized medicine and individualized dosing regimens.

IX. Conclusions and Standpoints

PKPD modeling and simulation of antibiotics is an underused technique that can help to increase the understanding of drug-bacteria interactions and improve development of dosing strategies. Because of the rapid increase in bacteria showing resistance to multiple of the currently available antibiotics, there is an urgent need to develop novel classes of antimicrobial agents with low potential for promoting resistance. Modeling and simulation can play a central role in making this process more efficient. However, we cannot rely only on the discovery of new antibiotics and must also pursue rational approaches to use and preserve the activity of the available antibiotics in the best way possible. In these cases, also, modeling and simulation is valuable in the search for regimens and drug combinations that optimize bacterial killing and minimize enrichment of subpopulations with lower drug sensitivity.

In this review, we have summarized pharmacometric models describing PKPD relationships for antibacterial effects of antibiotics. Most of the PKPD models available today are based on in vitro data as there is a lack of longitudinal data from animals and from clinical studies. However, we believe that much information can be obtained from in vitro models and that such models could be applied more frequently to compare dosing regimens and predict outcomes in patients and study designs. More effort is needed to implement and spread these techniques, and it is of vital importance that future models also consider resistance development.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Nielsen, Friberg.

References


