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Pharmacometric Models for Antibacterial Agents to Improve Dosing Strategies

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Abstract
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Antibiotics are among the most commonly prescribed drugs. Although the majority of these drugs were developed several decades ago, optimal dosage (dose, dosing interval and treatment duration) have still not been well defined. This thesis focuses on the development and evaluation of pharmacometric models that can be used as tools in the establishment of improved dosing strategies for novel and already clinically available antibacterial drugs.

Infectious diseases are common causes of death in preterm and term newborn infants. A population pharmacokinetic (PK) model for gentamicin was developed based on data from a prospective study. Body-weight and age (gestational and post-natal age) were found to be major factors contributing to variability in gentamicin clearance and therefore important patient characteristics to consider for improved dosing regimens.

A semi-mechanistic pharmacokinetic-pharmacodynamic (PKPD) model was also developed, to characterize *in vitro* bacterial growth and killing kinetics following exposure to six antibacterial drugs, representing a broad selection of mechanisms of action and PK as well as PD characteristics. The model performed well in describing a wide range of static and dynamic drug exposures and was easily applied to other bacterial strains and antibiotics. It is, therefore, likely to find application in early drug development programs.

Dosing of antibiotics is usually based on summary endpoints such as the PK/PD indices. Predictions based on the PKPD model showed that the commonly used PK/PD indices were well identified for all investigated drugs, supporting that models based on *in vitro* data can be predictive of antibacterial effects observed *in vivo.* However, the PK/PD indices were sensitive to the study conditions and were not always consistent between patient populations. The PK/PD indices may therefore extrapolate poorly across sub-populations. A semi-mechanistic modeling approach, utilizing the type of models described here, may thus have higher predictive value in a dose optimization tailored to specific patient populations.

Keywords: Pharmacometrics, pharmacokinetics, pharmacodynamics, modeling, NONMEM, antibiotics, *in vitro*, time-kill curve, PK/PD indices, gentamicin, aminoglycosides, newborn infants, premature infants, cystatin C

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Utan tvivel är man inte klok

Tage Danielsson

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

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II Nielsen, E.I., Cars, O., Friberg, L.E. Predicting *in vitro* antibacterial efficacy across experimental designs with a semimechanistic PKPD model. *Antimicrob Agents Chemother.* 31 January 2011;doi:10.1128/AAC.01286-10.

III Nielsen, E.I., Sandström, M., Honoré, P.H., Ewald, U., Friberg, L.E. Developmental pharmacokinetics of gentamicin in preterm and term neonates: population modelling of a prospective study. *Clin. Pharmacokinet.* 2009;48(4):253-63.

IV Mohamed, A.F., Nielsen, E.I., Cars, O., Friberg, L.E. A pharmacokinetic-pharmacodynamic model for gentamicin and its adaptive resistance with predictions of dosing schedules in newborn infants. *In manuscript.*

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Abbreviations

AR_{50}	Relative amount in AR_{ON} needed to reduce $E_{max(0)}$ by 50%
AR_{OFF}	Compartment for adaptive resistance being off
AR_{ON}	Compartment for adaptive resistance being on
AUBC	Area under the bacterial count curve
B_{max}	Bacterial count at stationary phase
C	Drug concentration
CDD	Case deletion diagnostics
C_E	Drug concentration in effect compartment
CFU	Colony-forming unit
CI	Confidence interval
CL	Clearance
COV	Covariate
$Cu_{ss,av}$	Average unbound plasma concentration at steady state
CV	Coefficient of variation
CXM	Cefuroxime
E_0	Baseline effect
EC_{50}	Drug concentration producing 50% of E_{max}
E_{max}	Maximum achievable effect
$E_{max(0)}$	Maximal achievable effect when no adaptive resistance has been developed
ERY	Erythromycin
EX_{50}	The magnitude of the PK/PD index required to achieve 50% of PD_{max}
*f*AUC	Area under the unbound plasma concentration-time curve
fC_{max}	Highest unbound plasma concentration
f_{mix1}	Fraction of experiments belonging to the first mixture
FOCE	First-order conditional estimation
f_{pers}	Fraction of bacteria in the R stage in the start inocula in the second mixture population
$fT_{>MIC}$	Cumulative percentage of the first 24 hrs of treatment with unbound drug concentrations exceeding the MIC
GA	Gestational age
GEN	Gentamicin
GFR	Glomerular filtration rate
IIV	Inter-individual variability
k_{death}	Rate constant for natural death of bacteria
k_{deg}	Rate constant for drug degradation

k_e	Rate constant for drug elimination
k_{e0}	Rate constant for effect delay
k_{growth}	Rate constant for multiplication of bacteria
k_{off}	Rate constant for reversal of adaptive resistance
k_{on}	Rate constant for development of adaptive resistance
k_{RS}	Rate constant for transformation from R to S
k_{SR}	Rate constant for transformation from S to R
L2	Level-two data item in NONMEM
LLP	Log-likelihood profiling
LOD	Limit of detection
LOQ	Limit of quantification
MAPE	Mean absolute prediction error
MIC	Minimum inhibitory concentration
MPE	Mean prediction error
MXF	Moxifloxacin
NLS	Non-linear least square algorithm
NPC	Numerical predictive check
OFV	Objective function value
P	Peripheral drug compartment
PAE	Post-antibiotic effect
PAE-SME	Post-antibiotic sub-MIC effect
PD	Pharmacodynamics
PD_{max}	Maximum achievable effect by a PK/PD index
PE	Prediction error
PEN	Benzylpenicillin
PI	Prediction interval
PK	Pharmacokinetics
PMA	Post-menstrual age
PNA	Post-natal age
PsN	Pearl-speaks-NONMEM
PWR	Allometric scaling exponent
Q	Inter-compartmental CL
R	Resting drug-insensitive bacterial population
R^2	Coefficient of determination
RSE	Relative standard error
S	Growing drug-sensitive bacterial population
SCM	Stepwise covariate model building
SME	Sub-MIC effect
TDM	Therapeutic drug monitoring
$T_{x\%}$	Time needed to reduce the start inocula by x%
V	Volume of distribution
VAN	Vancomycin
VPC	Visual predictive check
WT	Body-weight
XV	Cross validation

γ	Sigmoidicity factor characterizing the steepness of the concentration-effect relationship
ε	Difference between individual prediction and observation (residual error)
ε_{repl}	Replicate residual error
η	Difference between individual and population parameter estimate
θ	Typical population parameter value
τ	Dosing interval

Introduction

Treatment with antibacterial agents was first introduced in the late 1930s. This was a revolution in the management of infectious diseases and resulted in a reduction in overall morbidity and mortality [1-2]. During the following decades, there was great interest in this research area and several new classes of antibiotics were developed. Today, antibiotics are among the most commonly prescribed drugs and we rely on their effectiveness in the treatment of both mild and severe infections.

A combination of the development of resistance due to selective pressure from the extensive use of antibacterial agents and a decline in the development of new antibacterial agents has lead to a situation where we once again have bacteria causing infections that are no longer treatable. Since the 1970s, only two new classes of antibiotics (oxazolidinones and lipopeptides) have been approved [3-4]. Most pharmaceutical industries do not find the incentive to invest in this research area. Antibiotics are short-course therapies and therefore not as profitable as drugs to treat chronic or long-term conditions. The emergence of resistance also threatens to further shorten the life-span of new drugs on the market [2]. In U.S. hospitals each year, about 2 million people acquire bacterial infections and 90 000 die as a result [5]. In the EU, the situation is similar; up to 400 000 patients annually are reported to suffer from infections resistant to multiple antibiotics and more than 25 000 patients die from such infections [3]. Infectious diseases are once again considered to be a threat to global human health [6].

Appropriate antibiotic use means use only when needed. It also means that, when antibiotics are used, the choice of drug, dose, dosing interval and treatment duration should be evidence-based and tailored towards the intended patient to be treated. This thesis focuses on the development of pharmacometric models for antibacterial drugs with the aim to serve as tools in creating improved dosing strategies for novel as well as already clinically available antibacterial drugs. Model-based methods are increasingly used in drug development, providing a quantitative description of the time course of drug disposition and drug effects and toxicity [7-9]. Pharmacokinetic (PK) models describe the relationship between the dose of a drug and the resulting concentration at different sites of the body. Pharmacodynamic (PD) models describe the relationship between drug concentrations in plasma (or other fluids or tissues) and the resulting therapeutic effect or toxicity. Pharmacometric models are powerful tools to summarize and describe data that max-

imize the information gained from a study. More importantly, these models can provide for making inferences to new situations; for example in optimizing experimental setups, clinical trials and clinical drug use. A model-based strategy can lead to a more cost-effective drug development [7-9]. It can also provide for better dosing strategies, which would help to improve clinical outcomes in individual patients and hopefully also prolong the effective lifetime of pre-existing and newly developed antibiotics.

Role of PKPD in optimizing antibiotic dosing

When the effect of antibacterial agents is assessed in patients, differences in drug disposition, disease burden, immune defense and bacterial heterogeneity often contribute to high overall variability. Clinical data have therefore so far only supported rather simple PKPD models which relate clinical or microbiological outcomes to summary measures of drug exposure. However, for antibacterial drugs, the intended therapeutic effect is not exerted on the human body itself, but rather on a pathogen that is infecting the body. This offers the unique possibility of performing much of the PD characterization outside the human body. Therefore, *in vitro* and animal models play an essential role in the characterization of antibacterial drug effects.

Minimum inhibitory concentration (MIC)

Until 20 years ago, it was common practice to adjust the dosage of all antibacterial agents to obtain plasma concentrations that exceeded the MIC for as long as possible. The MIC is an *in vitro* measure, defined as the lowest static drug concentration that, after an incubation period of 16 to 20 hrs, completely inhibits visible growth in a start inoculum of approximately 5×10^5 colony-forming units (CFU)/ml. The MIC is the most widely used susceptibility marker for antibacterial drug effect. However, as a PD marker, the MIC has several disadvantages. It is measured as a threshold value and the effect of antibiotics is therefore often erroneously interpreted as a dichotomous variable, with drug concentrations exceeding the MIC resulting in full effect and with bacterial regrowth occurring as soon as the concentration falls below the MIC. Another drawback of the MIC is that it is a summary measurement of all the events that have taken place in the bacterial culture during the incubation period, including, for instance, drug degradation and the development of phenotypic and/or genetic resistance. Further, the MIC is usually measured using two-fold serial concentration dilutions, which have an inherently poor precision. Although the MIC is widely used to guide the choice of appropriate antibiotic treatment in the clinical setting and to detect changes in drug susceptibility, a more detailed characterization of the PD is warranted in the development of appropriate dosing regimens.

To compensate for some of the shortcomings of the MIC, additional terms such as the post-antibiotic effect (PAE), the sub-MIC effect (SME), and the post-antibiotic sub-MIC effect (PAE-SME) have been described [10-11]. The PAE is used to describe persistent suppression of bacterial growth after complete drug removal or after the drug concentration has declined below the MIC. The SME is the antibacterial effect of drug concentrations below the MIC and the PAE-SME refers to the increased PAE seen when bacteria in the PAE phase are re-exposed to sub-MIC concentrations. Although these measures are helpful in the characterization of the antibacterial effect, they are not easily translated into more optimal drug use.

In vitro time-kill studies

In time-kill studies, an *in vitro* start inoculum is exposed to antibacterial agents and sampled frequently for bacterial count measurements. During recent decades, various *in vitro* systems have been developed to complement the *in vivo* studies [12]. Although animal models have the advantage that they might provide similar growth conditions to those in humans, they have, apart from the ethical issues, the drawbacks of inter-species PK differences and a limit to number samples able to be collected [13-14]. The latter often means that only a point estimate of the PD measurement is provided from each animal, usually the bacterial count in the infected tissue after 24 hrs of drug exposure. Because of this, it is not known if the bacterial count is increasing, declining or static at the time of the observation and, therefore, animal studies often lack the ability to describe the time course of the effect. *In vitro* studies are easy to perform and they also allow more flexibility in the study design. The drug exposure in these experiments may be static, but it can also quite easily be modified to mimic different PK profiles and dosing regimens [15]. In addition, *in vitro* studies allow for more detailed PD characterization since they allow frequent quantification of the effect and thus enable the development of PKPD models that characterize the full time course of the effect.

Antibiotics have been categorized as having either a concentration- or time-dependent killing pattern, based on *in vitro* time-kill studies [16]. For concentration-dependent antibiotics, like the aminoglycosides and the fluoroquinolones, the bacterial killing is concentration-dependent over a wide range of concentrations [10]. For the time-dependent antibiotics, the maximal killing effect is reached at relatively low concentrations and increasing the concentration above 4–6 times the MIC will not increase the rate or extent of bacterial killing. This is a common characteristic of, for instance, the β-lactam antibiotics [10].

PK/PD indices

In recent decades, the activity of antibacterial drugs have also been classified according to the correlation to the three PK/PD indices: (i) the ratio of the highest unbound concentration to the MIC (fC_{max}/MIC), (ii) the ratio of the area under the unbound concentration-time curve to the MIC (fAUC/MIC), and (iii) the percentage of a 24 hr time period that the unbound concentration exceeds the MIC ($fT_{>MIC}$) [17].

A vast number of studies have been conducted using different *in vitro* and animal infection models with the aim of identifying which of these PK/PD indices best predicts the effect of various combinations of antibacterial agents and various pathogens (Table 1) [14, 18-21]. The three PK/PD indices are highly correlated and dose-fractionation studies are commonly used to discriminate between them. In these studies the same total daily dose is divided into smaller doses and administered at different dosing intervals. As a result of such studies, the activity of ß-lactam antibiotics has been classified as being mostly dependent on $fT_{>MIC}$ [18, 22-26], while the effect of the aminoglycosides and fluoroquinolones is correlated with either fC_{max}/MIC or fAUC/MIC [19, 25-27]. The PK/PD indices appear to differ among various agents of the glycopeptides and macrolides. In early studies, $fT_{>MIC}$ was reported to be the best predictor of erythromycin and vancomycin efficacy, based on their time-dependent killing pattern. However, more recent studies, using only clinically relevant dosing regimens, have indicated that the effect of these drugs is better correlated with fAUC/MIC [16, 26, 28].

It is not feasible to use a wide range of total daily doses and dosing intervals in clinical studies, which makes it difficult to differentiate between the predictive capacities of the PK/PD indices. Clinical studies have, however, been used to determine the magnitude of a predefined PK/PD index required to achieve a certain clinical or microbiological outcome [29-34]. After the establishment of the magnitude of the target PK/PD index required to achieve the desired antibacterial effect, simulations taking variability in PK and PD (in terms of MIC distribution) into account, has become a popular tool in guiding the selection of dosing regimens [35-37].

Table 1. *PK/PD indices most predictive of the effect for a selection of antibacterial drug classes.*

PK/PD index	Antibacterial drug class
fC_{max}/MIC	Aminoglycosides, fluoroquinolones
fAUC/MIC	Aminoglycosides, fluoroquinolones, macrolides, glycopeptides
$fT_{>MIC}$	β-lactams, (macrolides), (glycopeptides)

PKPD modeling for antibacterial agents

The PK/PD indices are summary endpoints which simplify a much more complex biological system. The design of dose schedules can be further optimized if based on models that take the whole time course of the PK and PD relationship into consideration. These models should provide for a separate quantitative description of the PK time course (i.e. drug concentration), the concentration-effect relationship (i.e. the effect that the drug imposes on the bacterial population) and the concentration-toxicity relationship. A modeling approach is generally considered to be more informative than a simple graphical approach or the use of correlation coefficients [38].

Mechanism-based PKPD models

The aim of creating PKPD models is generally to be able to describe and compare the effect of different drugs. More importantly, the aim is also to provide a tool that can be used for predictions that will be helpful in the development of improved dosing regimens in different patient populations. However, this requires the model to show good predictability even when it is applied to conditions other than those studied during its development. Incorporating prior knowledge and experience of the studied system into the model-building process to create mechanism-based PKPD models can increase the predictive ability of the model [39-40]. However, fully mechanism-based models are often too complex, and a semi-mechanistic approach is therefore often applied, where mechanistic knowledge is utilized but the model is kept simple enough to allow for parameter estimation.

Mechanism-based PKPD models should incorporate prior information about the bacterial system and the effects that drug impose on that system. It is common characteristics of time-kill curves that bacteria exposed to low or no concentrations of antibiotics grow exponentially until they approach a maximum bacterial level. At this high bacterial level, susceptibility to antibacterial agents is often significantly reduced, commonly referred to as the inoculum effect [41-42]. Exposure to higher concentrations of antibiotics often results in a biphasic killing curve, with the killing rate declining over time. This indicates the presence of persister cells, which are less susceptible to the antibiotics [43-45]. Until recently, little was known about the underlying mechanisms of persister cells. Increased knowledge of the production and nature of these cells could aid in the development of more mechanism-based PK/PD models.

Previously described PKPD models

Data from time-kill curve experiments have been used to support more or less complex semi-mechanistic PKPD models describing the time course of the effects of antibiotics [46]. The simplest semi-mechanistic PKPD model for antibacterial drugs involves a single bacterial compartment (B) with a

first-order rate constant for bacterial multiplication (k_{growth}) and a first-order rate constant for death of the bacteria (k_{death}), according to:

$$dB / dt = k_{growth} \cdot B - k_{death} \cdot B \qquad (1)$$

The antibacterial drug effect is most often included as an increase in the rate of bacterial death (k_{death}). This model structure has been shown to accurately describe *in vitro* time-kill curve data when only the first hours of the time-kill curve experiments are analyzed [47-49] or when *in vitro* kinetic systems are used to simulate declining drug concentrations, in which the regrowth of bacteria limits the ability to detect and characterize the presence of persister cells [48, 50-51]. A few PK/PD models have also been extended with regard to their semi-mechanistic complexities in order to describe the different growth and killing phases of a bacterial system. One approach has been to include a concentration- and/or time-dependent adaptation factor that influences either the growth constant (k_{growth}) [52] or the susceptibility, i.e. EC_{50}, of the bacteria [53-55]. The change in the killing rate over time has also been described in terms of true genetic heterogeneity of the total bacterial population in the start inocula [52, 56-59]. Models with several subpopulations with different drug-susceptibilities have also recently been used to characterize the inoculum effect, i.e. reduced killing at high bacterial counts [41, 57].

Gentamicin treatment in newborn infants

Infectious diseases, especially sepsis and pneumonia, along with preterm birth complications and birth asphyxia, are the main causes of death in the neonatal period [60]. In neonates, early warning symptoms of sepsis are often minimal, and the clinical course can rapidly become severe and even life-threatening. It is, therefore, important that antibacterial treatment of neonates with suspected sepsis is initiated without delay. It is, however, not always possible to take samples for culturing and the results from susceptibility testing are not always immediately available. The initial antibacterial therapy is therefore often empirical. *Escherichia coli* (*E. coli*) and other Enterobacteriaceae are among the most frequent Gram-negative pathogens, while Group B streptococcus and coagulase-negative staphylococcus are common Gram-positive pathogens [61]. Gentamicin in combination with a β-lactam antibiotic is therefore often recommended for empirical treatment of neonatal sepsis [61].

PK of gentamicin in newborns

The PK disposition of gentamicin in adults [62-63] as well as newborns [64] is multi-compartmental. Gentamicin, like other aminoglycosides, is highly hydrophilic. It is mainly distributed to extracellular water space, with a volume of distribution (V) in adults of approximately 0.3 L/kg [65]. Since the extracellular fluid volume is higher in newborns, especially if they are premature, V (per kilo body-weight, WT) for gentamicin is higher than in older children or adults. The extracellular body water accounts for as much as 65% of the total WT of the preterm neonate and 35-45% of the WT of the term neonate. As the child becomes older, the extracellular body water content decreases and, by one year of age, it has decreased to approximately 26-30% of the WT [66-67]. Gentamicin is eliminated almost exclusively by glomerular filtration [68]. The protein binding is low [69] and the clearance (CL) therefore mimics the glomerular filtration rate (GFR). The gentamicin CL is approximately 4 L/h in adults [70], but is considerably reduced in newborns because of anatomically and/or functionally immature kidneys.

Maturation of renal function

Nephrogenesis starts early, at approximately 5 weeks of gestation, and is completed at 36 weeks of gestation [71-72]. Because nephrogenesis is incomplete, GFR is lower in preterm neonates than in term neonates [67]. A correlation between the GFR and the gestational age (GA) has also been reported in several studies [73-75]. Even in term neonates, the GFR is low at birth. Birth triggers hemodynamic changes within the kidney, with resultant increased renal blood flow and decreased renal vascular resistance. The renal blood flow can be as low as 12 ml/min at birth, increasing to 140 ml/min at one year of age [67]. The maturation of the GFR is thus accelerated in the early post-natal period [73-74, 76-78]. WT corrected adult values for GFR are reached by 2.5 to 5 months after birth [67].

Markers of renal function

The gold standard for evaluation of GFR is to measure the urinary or plasma CL of exogenous markers, such as inulin, iohexol or isotopically labeled compounds [79]. However, these methods are expensive and time-consuming and endogenous markers are therefore often preferred in clinical practice. An ideal endogenous marker should be eliminated exclusively by glomerular filtration, have low protein binding and be easily measured. It is also of high importance that the marker has a stable production rate and that it is not affected by pathological changes [80]. The most commonly used endogenous marker for GFR is serum creatinine. However, creatinine has several limitations as a marker for GFR, such as being influenced by age, gender, muscle mass, and diet [81-82]. Another limiting factor when it is used in newborns is that creatinine crosses the placental barrier. The plasma

levels in neonates during the first post-natal period therefore reflect the maternal levels rather than neonatal renal function [83]. Cystatin C is an endogenous marker that has recently shown promising results as a marker for GFR [84-85]. Cystatin C is produced by most nucleated cell types and is freely filtered through the glomerular membrane and then re-absorbed and metabolised in the renal tubular cells [86]. The production rate of cystatin C in children seems to be independent of age and gender and, since it does not cross the placental barrier, cystatin C has the potential of being a useful renal marker in neonates [87-88]. However, its use in predicting drug elimination in the neonatal population has not yet been validated.

Allometric scaling

As described above, PK in newborn infants change with size (often described using WT) as well as the maturation of organ function. Thus, in order to show good predictive capacity, the PK model should preferably accommodate the effects of both size and maturity. One complicating factor is that the patient characteristics describing maturation (such as age) are highly correlated with size. Previously, the dose was commonly linearly scaled according to the WT [89]. Now, however, it is widely recognized that there is a non-linear correlation between WT and drug elimination and allometry is often used to describe this non-linearity according to:

$$P = a \cdot WT^{PWR} \qquad (2)$$

where P is the parameter of interest, a is the allometric coefficient and PWR is the allometric exponent. The allometric exponent is usually set as 1 for V and ¾ for CL [90-91]. Commonly, WT is included as a fixed primary covariate according to the allometry. This allows for other clinical characteristics that are highly correlated with WT, such as age, to be evaluated as secondary covariates independently of size [89].

PD of gentamicin

As for other aminoglycosides, gentamicin exhibits a concentration-dependent antibacterial effect with the presence of a PAE. The effect of gentamicin has been correlated either with fC$_{max}$/MIC or fAUC/MIC, with target breakpoints of 8–10 or 80–100, respectively [32, 92-94]. After initial exposure to gentamicin, the bacteria also show a period of adaptive or phenotypic resistance. This is a reversible form of resistance where short-term gentamicin exposures results in a transient period of reduced killing activity in an originally susceptible bacterial population. Adaptive resistance has been demonstrated in both *in vitro* and *in vivo* studies. The mechanism of this resistance is thought to involve down-regulation of the uptake of the aminog-

lycoside by the bacteria [95-97]. Adaptive resistance typically emerges soon after the first dose is administered and it is often hours or even days after drug removal before the bacteria regain full susceptibility [97].

The use of aminoglycosides is limited by their ability to cause nephrotoxicity and ototoxicity. Nephrotoxicity is caused by drug accumulation within the renal proximal tubule [98]. Animal studies have shown that the uptake, and thus the toxicity, of aminoglycosides is saturable in the clinically relevant concentration range [99] and also that continuous infusion regimens result in higher drug accumulation than intermittent dosing regimens using the same total daily dose [100]. In clinical studies, high trough levels have been associated with increased serum creatinine concentrations [101-102] and once-daily dosing regimens have been shown to be less nephrotoxic than twice-daily regimens involving the same total daily dose [103]. The relationship between ototoxicity and aminoglycoside levels is still not well characterized, but ototoxicity has been speculated to be correlated with the total aminoglycoside exposure [104]. The characteristics of concentration-dependent effect, PAE, and saturable renal uptake all promote the use of higher doses given less frequently. Understanding of how the development and reversal of adaptive resistance is influenced by the different dosing regimens and how to take adaptive resistance into account in order to improve dosing regimens is still limited.

Gentamicin dosing regimens in newborns

Historically, the dosing regimen of gentamicin involved multiple daily doses, typically of 2–2.5 mg/kg every 12–24 hrs. Lately, extended dosing intervals, based on the PD characteristics described above, are more widely used. Several meta-analyses have demonstrated either equivalence or superiority for extended-interval dosing regimens in terms of efficacy and nephrotoxicity [105-108]. In neonates, gentamicin dosing regimens currently involve doses of 4–5 mg/kg every 24–48 hrs initially, depending on GA, post-natal age (PNA) and/or WT [68]. Because of extensive, un-explained variability between and within individuals in the disposition of gentamicin, concentrations need to be closely monitored and dosing adjustments are frequently required in order to reach therapeutic levels. With an appropriate *a priori* individualized dosing regimen based on predictive covariates, therapeutic levels would hopefully be reached more quickly and the need for concentration monitoring could be reduced.

Although the PK of gentamicin in neonates have been extensively studied over the years, there is still no consensus on the most appropriate model (including the parameter-covariate relationships) to use in the design of optimal dosing regimens. Numerous retrospective studies based on data originally collected for routine therapeutic drug monitoring (TDM) have been described [109-115]. However, this type of data often contains insufficient

information to describe the known complex multi-compartmental PK of gentamicin [64]. A small and/or homogeneous study population also limits the power to identify covariates relevant for improved dosing regimens.

Based on the PD characteristics described above, the dosing recommendations for gentamicin are designed to reach a high peak concentration (>8–10 mg/L) together with a low trough concentration (<1–2 mg/L). These target concentrations will, however, always favor the use of extended dosing intervals. Because of the poor elimination capacity of neonates, dosing intervals as long as 60 hrs have recently been suggested [116]. However, when deciding on dosing recommendations, it should be remembered that the target concentrations are derived from regimens involving multiple daily doses, and prolonging the dosing interval too far will eventually allow surviving bacteria to re-grow between doses. *In vitro* time-kill studies simulating the neonatal concentration-time profile could provide better PD characterization in this patient sub-population.

Pharmacometrics

Pharmacometrics has been defined as "the science of developing and applying mathematical and statistical methods to characterize, understand, and predict a drug's PK, PD, and biomarker-outcome behavior" [117]. Pharmacometric models quantitatively characterize the relationships between drug dose, plasma concentration, biophase concentration (PK), and drug effects or side-effects (PD). The goal of these models is to summarize the data but, more importantly, also to reach a more thorough understanding of these processes and to predict future untested scenarios.

Mixed effects modeling

In mixed effects analysis, the data from all patients or all experiments are modeled simultaneously. With this approach, it is possible not only to describe the mean tendencies in the population (i.e. the typical values) but also to describe the random effects, i.e. the variability between and within individuals or experiments [118]. The advantages of this approach compared to the standard methodology, in which each individual's data are modeled separately, is that it allows relatively complex models to be estimated even if the data are sparse or imbalanced. If all data are analysed simultaneously and the study is appropriately designed, each individual patient or experiment can support different aspects of the model. This approach becomes especially valuable, for example, for neonates, where frequent sampling is not feasible [119].

The mixed effects approach also allows differentiation between variability between individuals/experiments (inter-individual variability, IIV) and vari-

ability originating from different types of errors, such as those related to dosing or sampling, bio-analytical errors and model misspecification. The parameter value in the individual (P_i) can be described according to:

$$P_i = \theta \cdot e^{\eta_i} \quad (3)$$

where θ is the typical value of the parameter in the studied population and η_i is the random effect describing the difference between the typical parameter value and the parameter value in the individual patient/experiment. Although the distribution of η (i.e. the parameter distribution in the population) may attain any shape, a log-normal parameter distribution (as in equation 3) is most often assumed. The residual error (ε_{ij}) describes the difference between an individual observation (y_{ij}) and the corresponding model prediction according to:

$$y_{ij} = f(X_{ij}, P_i) + \varepsilon_{ij} \quad (4)$$

where *f* symbolises a linear or non-linear function, P_i is a vector of individual model parameters and X_{ij} is a vector of independent variables, such as time or dose. The independent variables can also represent covariates. Covariates are typically patient characteristics such as weight, age or kidney function which, when introduced into the model, are able to reduce the unexplained variability. As for η, the residual error (ε) may attain any shape. Equation 4 corresponds to an additive residual error model, but proportional or combined additive and proportional residual error models are also frequently used.

Commonly, the model is evaluated in term of its ability to characterise the same data that were used in its development (internal validation). Although of high importance, in forecasting new patients or new types of studies or experiments, internal validation could potentially provide an over-optimistic projection. A valuable complement is therefore to perform the evaluation using data from a new set of individuals or experiments, i.e. to perform an external validation.

Aims

The general aim of the thesis was to develop predictive pharmacometric models for antibacterial agents to provide a better understanding of PK and PD processes as a basis for improved dosing strategies.

The specific aims were:

- To develop semi-mechanistic PKPD models to describe the time course of effects observed in *in vitro* bacterial cultures exposed to antibacterial agents with different mechanisms of action.

- To evaluate the performance of the developed PKPD model in (i) predicting antibacterial effects across experimental designs and (ii) predicting the currently used PK/PD indices.

- To describe the PK of gentamicin in preterm and term newborn infants in a prospective clinical study and to evaluate different dosing regimens based on previously used therapeutic targets as well as a semi-mechanistic PKPD model.

Materials and Methods

In vitro data

Bacterial strains and media

Streptococcus pyogenes group A, M12 NCTC (National Culture Type Collection) P1800 was used as test organism in Papers I and II. In Paper IV, the test organism was *E. coli* ATCC (American Type Culture Collection) 25922.

Todd-Hewitt broth was used as liquid medium for experiments with *S. pyogenes*, and Mueller Hinton broth supplemented with Ca^{2+} and Mg^{2+} was used for experiments with *E. coli*. The bacteria were seeded on blood agar plates (Columbia agar base with 5% horse blood) for viable count measurements.

Antibiotics and MICs

The following six antibacterial drugs were used in the *in vitro* studies: benzylpenicillin (Bensylpenicillin AstraZeneca®, AstraZeneca), cefuroxime (Zinacef®, GlaxoSmithKline), erythromycin (Abboticin®, Abbott), gentamicin (Sigma-Aldrich), moxifloxacin (Bayer) and vancomycin (Vancomycin Abbott®, Electra-Box Pharma) (Table 2). Fresh stock solutions were prepared prior to each experiment by dissolving the antibiotic in sterile distilled water (benzylpenicillin, cefuroxime, erythromycin, gentamicin, and moxifloxacin) or sterile phosphate buffer saline (vancomycin). The desired concentrations were obtained after appropriate dilutions in liquid medium.

The MIC values for benzylpenicillin, erythromycin, moxifloxacin and vancomycin were determined by E-tests (Biodisk AB, Solna, Sweden). For cefuroxime and gentamicin, the MIC was determined by the macrodilution technique, according to the guidelines of the Clinical and Laboratory Standards Institute (formerly NCCLS). The MIC was determined in at least triplicate on separate occasions. The MICs for *S. pyogenes* were 0.012, 0.0313, 0.125, 0.125 and 0.25 mg/L for benzylpenicillin, cefuroxime, erythromycin, moxifloxacin and vancomycin, respectively. The MIC of gentamicin for *E. coli* was 2 mg/L.

Time-kill curve experiments

In the time-kill curve experiments, the culture vessel was filled with liquid medium and bacteria from a 6 h logarithmic growth phase culture were added to obtain a start inoculum of approximately 10^6 CFU/ml. Antibiotics were added to achieve the desired start concentrations and the experiments were incubated at 35ºC for 24 or 48 hrs, during which samples were taken frequently for viable count measurements. The samples were serially diluted and plated onto two to four blood agar plates. After incubation at 35°C for 18 to 24 hrs, the CFUs were counted manually. The limit of detection (LOD) was 10 CFU/ml and drug carry-over was assessed by visual inspection of the distribution of colonies on the plates. Each time-kill experiment was carried out in at least duplicate on separate occasions. Growth control experiments (i.e. experiments without addition of antibiotics) were included for both the static and dynamic experiments. For the growth control experiments, start inocula lower than 10^6 CFU/ml were also used in order to fully characterize the growth kinetics. All viable counts were transformed into natural logarithms before data analysis. A summary of the time-kill curve experiments performed in this thesis (with experimental type, initial concentrations and simulated half-lives) is shown in Table 2.

Static time-kill curve experiments

The static time-kill curve experiments were performed using 10 ml glass tubes with a total broth volume of 4 ml. The tubes were placed in sand to minimize fluctuations in the temperature during the experiments.

Dynamic time-kill curve experiments

A previously described *in vitro* kinetic system was used to perform the dynamic time-kill curve experiments [15, 24]. In this system, an open-bottomed spinner flask (110 ml) is connected to a pump which removes medium at a constant rate (*Figure 1*). This creates negative pressure in the culture vessel and fresh medium is sucked into the vessel, resulting in a first-order elimination rate of the antibiotic. A filter membrane and a pre-filter are placed between the base and the flask to prevent dilution of the bacteria and thus keep the system closed. A magnetic stirrer is placed above the membrane to prevent membrane blockage and to ensure homogeneous mixing. One side arm of the spinner flask is connected to a vessel containing the fresh medium to be drawn into the flask. A silicone membrane is inserted into the other side arm to enable repeated sampling.

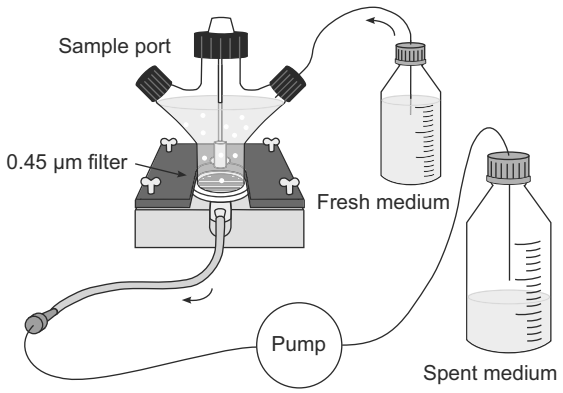

Figure 1. Schematic illustration of the *in vitro* kinetic system used for the dynamic time-kill curve experiments.

Antibiotic stability

The stability of the antibiotics during the incubation period was tested in separate time-kill curve experiments at two different initial concentrations. The antibiotic concentrations were analyzed using the microbiological agar diffusion method (Paper I) or fluorescence polarization immunoassay (Paper IV) and the degradation was assumed to follow first-order kinetics.

Table 2. *Summary of the time-kill curve experiments performed in the thesis*

Drug	Type	Initial Concentrations[a] (τ)	Sim $t_{1/2}$ (h)	Paper
Benzylpenicillin	Static	0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 16, 64	-	I
	Dynamic	2, 16	0, 1.0, 3.0	II
	Repeated	2 (4 h), 16 (11 h)	1.0	II
Cefuroxime	Static	0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 16, 64	-	I
	Dynamic	2, 16	0, 1.7, 5.1	II
Erythromycin	Static	0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 16, 64	-	I
	Dynamic	2, 16	0, 1.7, 5.1	II
Gentamicin	Static	0.0625, 0.125, 0.25, 0.5, 1, 2, 8	-	IV
	Dynamic	1.0, 2.0, 3.9, 8	2.1[b], 19.1[c]	IV
	Repeated	2.0 (6 h,12 h), 3.9 (24 h), 8 (24 h)	2.1[b], 19.1[c]	IV
Moxifloxacin	Static	0.25, 0.5, 1, 2, 4, 16, 64	-	I
	Dynamic	2, 16	0, 12.7, 38.1	II
Vancomycin	Static	0.25, 0.5, 1, 1.5, 2, 4, 16, 64	-	I
	Dynamic	2, 16	0, 5.1, 15.2	II

[a] as multiples of MIC
[b] corresponding to the first flow-rate for gentamicin two-compartment kinetics
[c] corresponding to the second flow-rate for gentamicin two-compartment kinetics

Clinical data

A prospective clinical study was performed in the Neonatal Intensive Care Unit (NICU) at the University Children's hospital, Uppsala, Sweden, with the aim of characterizing the population PK of gentamicin in preterm and term newborn infants. Approval was obtained from the Regional Ethics Committee of Uppsala University, Sweden, and the Swedish Medical Product Agency. All parents gave their written informed consent prior to inclusion.

Patients

All neonates receiving gentamicin in the NICU between March 2005 and September 2006 were eligible to be included in the study. Gentamicin (Garamycin®, Schering-Plough) was administered intravenously as short infusions over 5 minutes at an initial dosing of 4 mg/kg every 24 hours. The maintenance dosing regimen was adjusted according to routine TDM sampling in order to achieve peak concentrations (1 hr post-infusion) of 8–10 mg/L and trough concentrations of <2 mg/L. In addition to the routine TDM samples, an average of four extra study samples were taken per individual for gentamicin analysis. The extra study samples were taken on treatment days 2 and 5 and included one sample taken approximately 15 minutes after the end of the infusion and one sample taken 4–8 hrs after the end of the infusion. Samples for analysis of creatinine and cystatin C concentrations were taken on treatment days 1, 2 and 5. If a patient included in the study received additional gentamicin treatment while in the NICU, the TDM data related to this treatment period were collected and included in the analysis.

Chemical assays

All chemical assays related to the *in vivo* study were performed at the Department of Clinical Chemistry, University Hospital, Uppsala. Concentrations of gentamicin in serum were analyzed using fluorescence polarization immunoassay technique (TDx, Abbott Laboratories). The limit of quantification (LOQ) was 0.3 mg/L and the coefficient of variation (CV) was <7%. Plasma creatinine levels were measured using the modified kinetic Jaffe reaction (LOQ 18 μmol/L, CV <3.2%). Plasma cystatin C concentrations were analysed using immunoassays (LOQ 0.3 mg/L, CV <4.8%) [120-121].

Model development

The model development was performed using NONMEM and all available data within each paper were modeled simultaneously.

Semi-mechanistic PKPD model (Papers I–II, IV)

As a first step, a general semi-mechanistic PKPD model was developed based on static time-kill curve experiments where an *S. Pyogenes* strain was exposed to a wide range of constant concentrations of the five antibiotics: benzylpenicillin, cefuroxime, erythromycin, moxifloxacin and vancomycin (Table 2, Paper I). In the second step, a set of dynamic time-kill curve experiments was carried out to evaluate the ability of the PKPD model to predict the time course of the antibacterial effects when the concentration of the antibiotic decreased over time, using similar half-lives to those observed in patients (Table 2, Paper II). The model was then developed further to characterize the bactericidal activity of a range of gentamicin exposures, both static and dynamic, on a strain of *E. coli* (Table 2, Paper IV). The PKPD model was expanded in this step to characterize the development and reversal of phenotypic or adaptive resistance.

Structural model

The concentration of the antibiotic (C) was constant, decreasing because of drug instability (k_{deg}) or decreasing according to an elimination rate (k_e), as simulated in the *in vitro* kinetic system (equation 5). The degradation rate was determined in separate time-kill curve experiments and was assumed to follow first-order kinetics. An effect compartment model was used to allow for a possible time delay between adding the drug and observing an effect in terms of enhanced bacterial killing (equation 6). The effect delay was characterized by a first-order rate constant (k_{e0}) and the effect compartment (C_E) was introduced without affecting the mass balance of the kinetic compartment (C) [122].

$$dC / dt = -k_{\text{deg}} \cdot C - k_e \cdot C \quad (5)$$

$$dC_E / dt = k_{e0} \cdot C - k_{e0} \cdot C_E \quad (6)$$

In this model, in order to describe the decrease in net growth rate when the system is approaching the stationary phase, the bacterial system was described by implementing the idea of phenotypic switching between normally growing cells and persister cells with reduced growth rates [43-45]. A model in which the total bacterial population was divided into two subpopulations, one growing population (S) and one resting population (R), was used (equa-

tions 7–8). In the early logarithmic growth phase, most of the bacteria were assumed to be in the growing stage (S), with the net growth rate determined by the rate constant for multiplication of bacteria (k_{growth}) and the rate constant for natural death of bacteria (k_{death}). In the resting stage (R), the bacteria show no growth, although they were still assumed to have the same rate of natural death as the bacteria in the growing stage.

$$dS/dt = k_{growth} \cdot S - k_{death} \cdot S - k_{SR} \cdot S + k_{RS} \cdot R \quad (7)$$

$$dR/dt = -k_{death} \cdot R + k_{SR} \cdot S - k_{RS} \cdot R \quad (8)$$

It was assumed that bacteria in the growing stage would be transformed into the resting stage when the total bacterial content in the system reached high values, with a rate constant k_{SR}. It was found that k_{SR} could be well described using a linear function with a proportionality constant multiplied by the total bacterial content in the system (S+R). Instead of estimating this proportionality constant, the model was re-parameterized to estimate the more easily comprehensible parameter B_{max}, which is the number of bacteria in the stationary phase (equation 9).

$$k_{SR} = \frac{(k_{growth} - k_{death})}{B_{\max}} \cdot (S + R) \quad (9)$$

The experiments included in this thesis were not designed to characterize the reversibility of the formation of persister cells. The transfer from the resting stage into the sensitive stage (k_{RS}) was considered to be negligible in these experiments and was set at 0.

It was also assumed that the antibacterial drugs would only affect the bacteria in the growing stage. With this assumption, the presence of persister cells also characterizes the reduced drug-sensitivity at high bacterial counts as well as the biphasic killing behavior often seen in time-kill curve experiments. The antibacterial effect was assumed to be non-linearly dependent on the drug concentration in the effect compartment (C_E) and was modeled using a sigmoidal E_{max} model (equation 10) where E_{max} is the maximum achievable killing rate of the bacteria in the susceptible stage, EC_{50} is the concentration of the antibiotic that results in 50% of E_{max}, and γ is the sigmoidicity factor characterizing the steepness of the concentration-effect relationship. Simple E_{max} models (γ set to 1) and linear models were also evaluated. It was assumed that the drug effect would be an increase in the death of the bacteria in the growing drug-susceptible population and this drug effect was incorporated into equation 7 according to equation 11.

$$DRUG = \frac{E_{\max} \cdot {C_E}^{\gamma}}{{C_E}^{\gamma} + EC_{50}^{\gamma}} \quad (10)$$

$$dS/dt = k_{growth} \cdot S - (k_{death} + DRUG) \cdot S - k_{SR} \cdot S + k_{RS} \cdot R \quad (11)$$

Adaptive resistance of gentamicin

The development of adaptive resistance on exposure to gentamicin was modeled using a binding function, where the degree of binding resulted in a reduction in E_{max} (equations 12–14). The degree of reduction in E_{max} was determined by a relative fraction that was transferred from a compartment representing adaptive resistance being turned off (AR_{OFF}) to a compartment with adaptive resistance being turned on (AR_{ON}). At the start of the experiments the relative fraction was 1 in AR_{OFF} and 0 in AR_{ON}. The gentamicin concentration (C_E) was driving the transfer from AR_{OFF} to AR_{ON}. k_{on} and k_{off} describe the rates of development and reversal of adaptive resistance, respectively. $E_{max(0)}$ represents the maximum achievable rate constant for bacterial killing when no adaptive resistance has developed and AR_{50} is the relative amount in the compartment for adaptive resistance (AR_{ON}) needed to reduce $E_{max(0)}$ by 50%.

$$dAR_{ON}/dt = k_{on} \cdot AR_{OFF} \cdot C_E - k_{off} \cdot AR_{ON} \quad (12)$$

$$dAR_{OFF}/dt = -k_{on} \cdot AR_{OFF} \cdot C_E + k_{off} \cdot AR_{ON} \quad (13)$$

$$E_{\max} = E_{\max(0)} \cdot \left(1 - \frac{AR_{ON}}{AR_{ON} + AR_{50}}\right) \quad (14)$$

Models for random effects

Inter-experimental variability was not estimated. However, the mixture module in NONMEM was used to allow for some variability in the proportion of bacteria in the resting stage in the start inocula (Papers I–II). Two subpopulations of experiments were allowed. In the first subpopulation, no resting bacteria were present in the start inocula. The proportion of the total number of experiments belonging to the first subpopulation (f_{mix1}) and the fraction of bacteria in the resting stage (R) in the start inocula in the second subpopulation (f_{pers}) were estimated as parameters.

Additive residual error on the natural logarithmic scale was applied. Since each sample was plated onto several plates and all raw data from all evaluated plates were included in the data analysis, there was more than one observation per sampling time point. To avoid bias that may occur as a result

of correlations between these replicate samples, the residual error was divided into and estimated as two components: one common residual error (ε) and one replicate-specific error (ε_{repl}) [123]. The level-two (L2) data item in NONMEM was used to group the replicate measurements together.

Population PK of gentamicin in newborn infants (Paper III)

Basic model

One-, two- and three-compartment models were fitted to untransformed or ln-transformed gentamicin concentration data. Inter-individual variability, covariance and inter-occasion variability in model parameters were allowed and assumed to be log-normally distributed. The residual error was modeled using additive, proportional or combined additive and proportional error models.

Covariate model

In neonates, both size and organ maturation have a profound influence on the PK profile. Covariates representing size and maturation are, however, often highly correlated and this needs to be addressed in the analysis. In this study, size (represented by WT) was included in all model parameters as the primary covariate, using allometric scaling according to:

$$P = \theta_1 \cdot WT^{PWR} \quad (15)$$

where θ_1 represents the typical value of the parameter P in a neonate weighing 1 kg, and PWR is the allometric scaling parameter, set to ¾ for CL and inter-compartmental clearances (Q) and 1 for V [90, 124-125]. Additional covariates were included in a stepwise fashion, with forward inclusion and backward deletion using automated stepwise covariate model building (SCM) [126]. Categorical relationships were introduced according to:

$$P = \theta_1 \times (1 + \theta_2 \times COV) \quad (16)$$

where θ_1 is the population estimate for category 1 and θ_2 is the fractional difference in P between categories 1 and 2. Continuous covariate relationships were first introduced in a linear fashion according to:

$$P = \theta_1 \times (1 + \theta_2 \times (COV - COV_{median})) \quad (17)$$

where θ_1 is the typical value for the parameter P in a patient with the median covariate value (COV_{median}) and θ_2 is the fractional change in P with each unit deviation from COV_{median}. If a covariate was included in a linear fashion,

non-linear inclusion (e.g. power, exponential or piece-wise linear relations) was also tested.

Only covariates that were regarded as likely to have an essential impact on a parameter were considered. Investigated covariates included: WT, PNA, GA, post-menstrual age (PMA), creatinine, 1/creatinine, cystatin C, 1/cystatin C, GFR calculated using cystatin C [121], and concomitant medication with potentially nephrotoxic drugs (cefuroxime, vancomycin or indomethacin). All three of the covariates describing age, i.e. GA, PNA and PMA, were considered for inclusion in the first step of the covariate analysis. However, PMA was not considered for inclusion if PNA or GA were already in the model. Similarly, GA or PNA were not investigated if PMA was previously included.

Handling data below the LOD and LOQ

The way in which data below the LOD or LOQ are handled can influence the results [127]. In Paper I, only a small fraction of the data were <LOD and only the first value in a consecutive series of values <LOD were entered in the dataset (as LOD/2) and all other values <LOD were omitted. In Papers II and IV, a larger fraction of the observations were <LOD. In these studies, the M3 method was used; in which all observation time-points were retained in the analysis, and data <LOD were handled using a likelihood-based approach, including an estimation of the probability of observing data <LOD for these observation records. In Paper III, only one value was <LOQ and this value was omitted from the analysis.

Model selection

The modeling process was guided based on several criteria including: mechanistic plausibility, the objective function value (OFV) supplied by NONMEM, the precision of the parameter estimates, and evaluation of graphical diagnostic plots. The likelihood ratio test was used to evaluate statistically significant differences between nested models. The difference in OFV approximately follows a χ^2 distribution with the number of degrees of freedom corresponding to the difference in the number of parameters between the full and reduced models [128-129]. As an example, for a statistical significance level of $p<0.001$, the more complex model (one extra parameter) was chosen if the reduction in OFV was at least 10.83. In the covariate analysis in Paper III, the importance of retaining a covariate was also assessed from the magnitude of the covariate effect and the magnitude of reduction in inter-individual and residual variability.

Evaluation of model performance

Internal model evaluation (Papers I–IV)

Simulation-based diagnostics are currently frequently used for internal evaluation of pharmacometric models [130]. Summary statistics, such as median and prediction intervals (PI), are calculated based on simulations from the model using the original dataset as template. The numeric predictive check (NPC) evaluates the appropriateness of the model by numerically presenting the number of observations falling within the prediction intervals (PI). With the visual predictive check (VPC), a graphical comparison of the distribution (median and PIs) of the simulated data and the observed data is performed.

The PKPD model developed in Paper I was evaluated using internal model validation with cross validation (XV) and case-deletion diagnostics (CDD). During the XV, data from experiments using the same drug concentration were excluded one by one and the model parameters were re-estimated based on the remaining data. The excluded experiments were then predicted using the re-estimated parameter values. In the CDD, data from either one day of experiments or one single experiment were excluded from the full dataset. The parameter values were then re-estimated and compared with the estimates from the model based on the full dataset.

External model evaluation (Paper III)

External model validation was used to assess the predictive performance of the population PK model developed in Paper III. An external evaluation dataset was used, comprising 270 gentamicin concentration measurements (96 peak concentrations, 154 trough concentrations and 20 mid-point samples) from 113 neonates collected in a previous study [110]. The predictive performance was assessed by applying the final model, without parameter re-estimation, to the evaluation dataset. For each observation in the evaluation dataset, the prediction error (PE) was assessed based on the population predicted concentration (according to $100\times(C_{pred}-C_{obs})/C_{obs}$). Bias and precision were assessed in terms of the mean prediction error (MPE) and the mean absolute prediction error (MAPE) [131].

Model performance across experimental designs (Paper II)

The performance of the developed semi-mechanistic PKPD model (Paper I) in describing and predicting observed bacterial counts within and across experimental designs was assessed using simulation-based diagnostics. The observed data (static and dynamic) were compared to model-simulations where the parameter estimates were obtained using three different sets of data: (i) static experiments, (ii) dynamic experiments, (iii) combined static

and dynamic experiments. The combined dataset (iii) was used as template, and 1000 simulated datasets were generated for each of the three scenarios (i.e. parameters estimated using the datasets i–iii). The observed data were compared with the distribution (median and 95% PI) of the simulated data and presented graphically as VPCs. The impact of differences in experimental setup between the static and dynamic experiments was also investigated using SCM, where the setup (i.e. static or dynamic experiments) was introduced as a potential categorical covariate (equation 16) for each estimated parameter in the model.

Predicting *in vivo* PK/PD indices (Paper V)

The developed PKPD model was evaluated for its capacity to predict the previously determined PK/PD indices for the six drugs investigated in the thesis. To evaluate and challenge the methodology used in the establishment of the PK/PD indices, the sensitivity to factors such as different PK characteristics, uncertainty in MIC, and choice of PD endpoint were also investigated by altering the simulation settings.

Simulated dosing regimens

A dose-fractionation study was simulated for each of the six drugs. A wide range of total daily doses (n=16) was simulated to be administered as constant drug exposures or with dosing intervals of 4, 8, 12 and 24 hrs. The simulated doses corresponded to the daily doses needed to achieve an average unbound plasma concentration at steady state ($Cu_{ss,av}$) of 0.0078–256×MIC for each respective drug. In the simulations, benzylpenicillin, cefuroxime and gentamicin were administered as intravenous bolus injections and erythromycin, moxifloxacin and vancomycin were administered as continuous intravenous infusions over 60 min. For each drug, a common clinically used total daily adult dose was also included. These were as follows: 6000 mg, 2250 mg, 1200 mg, 315 mg, 400 mg, and 2000 mg daily for benzylpencillin, cefuroxime, erythromycin, gentamicin, moxifloxacin and vancomycin, respectively.

Default simulation setting

PK models were searched for in the literature and combined with the semi-mechanistic PKPD model developed in Papers I–II and IV. For benzylpenicillin and gentamicin, three-compartment PK models were used to simulate the human concentration-time profile [63, 132]. For cefuroxime, erythromycin, moxifloxacin and vancomycin, two-compartment PK models were used [133-136]. The combined models were used to predict the bacterial killing kinetics in a start inoculum of 10^6 CFU/ml following the dosing regimens described above. The bacterial count after 24 hrs of drug exposure was used as the PD endpoint.

Altered simulation settings

- *Modification of PK.* Predictions were performed after (i) CL was reduced to 1/3 of its value in the default setting and (ii) the PK model was altered to characterize the concentration-time profile for benzylpenicillin and gentamicin in preterm neonates (GA 30 weeks, PNA 2 days) [137-138].
- *Uncertainty in MIC.* The sensitivity to the measured MIC value was investigated by changing the MIC to (i) one step-dilution higher or lower, i.e. 0.5×MIC and 2×MIC, (ii) the estimated EC_{50} value, and iii) an MIC value calculated from the *in vitro* PKPD model, i.e. the static concentration (adjusted for *in vitro* drug degradation) that, after an incubation period of 20 hrs, resulted in a bacterial count of 10^7 CFU/ml using a start inoculum of 5×10^5 CFU/ml.
- *Alternative PD endpoints.* In addition to the bacterial count after 24 hrs of treatment, the following alternative PD endpoints were also investigated: (i) the bacterial count after 12, 48, and 240 hrs of exposure; (ii) the time needed for the initial inocula to be reduced 100-fold ($T_{99\%}$), 1 000-fold ($T_{99.9\%}$) or 10 000-fold ($T_{99.99\%}$); and (iii) the area under the $\log_{10}$ of the bacterial count curve from 0 to 24 hrs ($AUBC_{24}$) or 0 to 48 hrs ($AUBC_{48}$) [20, 139].
- *Modification of the concentration driving the antibacterial effect.* The relationship between the antibacterial effect and the PK/PD indices was also assessed by letting the concentration in one of the peripheral compartments (instead of the central PK compartment) driving the antibacterial effect.

Data evaluation

The three PK/PD indices (fC_{max}/MIC, fAUC/MIC, and $fT_{>MIC}$) were determined for each dosing regimen and each of the six drugs [17]. The relationship between the effect and the PK/PD indices was evaluated according to:

$$E = E_0 - \frac{PD_{max} \cdot X^{\gamma}}{X^{\gamma} + EX_{50}^{\gamma}} \qquad (18)$$

where E is the PD endpoint, E_0 is the baseline effect representing the value of E without drug treatment, X is the value of one of the three PK/PD indices, PD_{max} is the maximum effect, EX_{50} is the magnitude of X required to achieve 50% of E_{max}, and γ is the sigmoidicity factor, reflecting the steepness of the relationship. Each drug and each PK/PD index was fitted separately but simultaneously for all data from all dosing regimens. The coefficients of determination (R^2), the log-likelihood value and a visual inspection of the graphs were used in the selection of the PK/PD index that best predicted the antibacterial effect. The magnitude of the selected PK/PD index required to

achieve a bacteriostatic or bactericidal effect was calculated, where a bacteriostatic effect was defined as an unchanged bacterial count at 24 hrs compared with the initial inocula, and a bactericidal effect was defined as a 3 $\log_{10}$ decrease in bacterial count (99.9% killing) at 24 hrs compared with the initial inocula. These simulations were performed without variability, i.e. the simulations were predictions based on typical structural model parameters.

Prediction of gentamicin dosing regimens

Gentamicin concentration-time profiles were predicted by the PK model developed in Paper III and initially evaluated according the traditionally used therapeutic target concentrations. It was assumed that the gentamicin treatments were started on the day of birth and they included four dosing regimens with doses of 4–5 mg/kg (as 5 min infusions) given with dosing intervals of 24–48 hrs. The PK profiles were predicted for four typical study individuals with GAs of 25, 29, 34 and 40 weeks and birth weights of 778, 1200, 2710 and 3500 g, respectively. Further, for all studied neonates with weight measurements during the first post-natal week, the same four dosing regimens were simulated (N=1000) based on the final model using the individual covariates. The 10^{th}, 50^{th}, and 90^{th} percentiles of the simulated trough and peak (1 hr post-infusion) gentamicin concentrations were calculated after the first dose and after one week of treatment for each neonate and plotted against the GA.

The change in bacterial count over time was also predicted by combining the PK model in Paper III with the PKPD model developed in Paper IV. The covariate characteristics of the four typical study individuals described above were used in the predictions. Also here, it was assumed that the treatments would start on the day of birth, and doses of 2–7 mg/kg (as 5 min infusions) administered at dosing intervals of 8–48 hrs were included. It was also assumed that the drug concentration in the central PK compartment was driving the antibacterial effect, i.e. resembling systemic bacteremia, with the pathogen located in the plasma compartment.

Software

Modeling and simulations were performed using the first-order conditional estimation (FOCE) method (Papers I, III), or the Laplacian method (Papers II, IV, V) in the population analysis software NONMEM, version VIβ or VI [129]. The Xpose program [140] (xpose.sourceforge.net, accessed 2011/02/05), S-Plus (Insightful Corp), and R (www.r-project.org, accessed 2011/02/05) were used in graphical, statistical and explanatory analyses. Pearl-speaks-NONMEM (PsN) was used for SCM, nonparametric boot-

straps, simulations and calculations for NPC and VPC, and for the execution of log-likelihood profiling (LLP) [126, 141](psn.sourceforge.net, accessed 2011/02/05). In Paper V, R was used to visualize the data, perform non-linear curve fitting (nls) and calculate the coefficients of determination (R^2) and log likelihood.

Results

Semi-mechanistic PKPD model

A semi-mechanistic PKPD model was developed to describe bacterial growth and killing kinetics in *in vitro* bacterial cultures following a wide range of exposures to six antibacterial drugs with different mechanisms of action. A schematic illustration of the final model is shown in *Figure 2*. This figure represents the full model. Reductions were made when the model was applied to subsets of the data. For instance, the PK component was reduced to a single central drug compartment when the model was applied to the data from the time-kill curves. Similarly, the part describing adaptive resistance was only used when there was an indication of such resistance in the data.

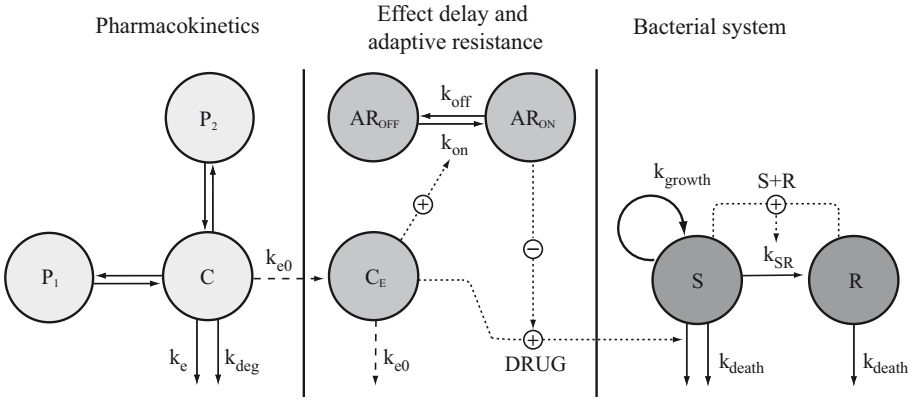

Figure 2. Schematic illustration of the full PKPD model describing the time course of drug concentration, bacterial growth and killing after antibacterial treatment, and the development and reversal of adaptive resistance. C, central drug compartment; P_1 and P_2, peripheral drug compartments (used for simulation purposes); C_E, drug effect compartment; AR_{OFF} and AR_{ON}, compartments describing adaptive resistance (used for gentamicin); S, proliferating, drug-sensitive bacteria; R, resting, drug-insensitive bacteria; k_e, drug elimination rate constant; k_{deg}, rate constant for drug degradation; k_{e0}, rate constant for effect delay; k_{on} and k_{off}, rate constants for development and reversal of adaptive resistance, respectively; k_{growth}, and k_{death}, rate constants for multiplication and degradation of bacteria, respectively; k_{SR}, rate constant for transformation from the growing, sensitive stage into the resting stage.

General PKPD model (Papers I–II)

Representative time-kill curves for the static experiments used in the initial development of the PKPD model are shown in *Figure 3*. In these experiments, an *S. pyogenes* strain was exposed to a wide range of constant concentrations of the five antibiotics: benzylpenicillin, cefuroxime, erythromycin, moxifloxacin and vancomycin. As expected, bacteria exposed to no antibiotics or low concentrations of antibiotics grew exponentially until high bacterial counts were reached and a stationary level was approached (*Figure 3*). At higher drug concentrations, the killing curve was biphasic, with a decline in the killing rate over time, for all antibiotics except vancomycin. Vancomycin only produced a modest antibacterial effect and showed indications of an all or nothing effect. For the same bacterial strain and antibiotics, a second set of experiments with dynamic concentration-time profiles were performed (*Figure 4, right panel*) with varying start concentrations (2 and 16 ×MIC) and elimination conditions (human half-life, reduced half-life and constant concentrations). In these experiments, the initial bacterial killing was typically followed by bacterial regrowth as the drug concentration declined.

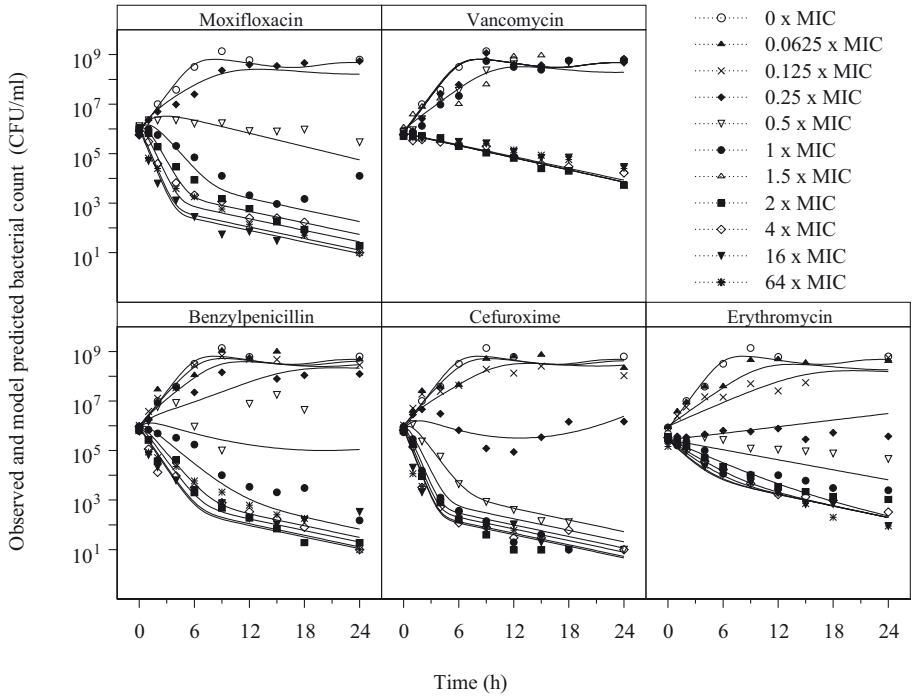

Figure 3. Time-kill curves for *S. pyogenes* after exposure to five antibiotics at static concentrations ranging from 0–64 times the MIC. Data from single experiments are shown and the lines represent model predictions using estimation based on the static data alone.

When the static and dynamic time-kill curve data were analyzed separately, the final PKPD model described both types of data well for all five studied antibiotics. As shown in the VPCs in *Figure 4*, the final model also adequately described both the static and dynamic data when they were analyzed simultaneously. However, with the simultaneous analysis, the model slightly under-predicted the effects of vancomycin in some of the dynamic experiments (*Figure 4, top panel*). In contrast, the model over-predicted the effects towards the end of the experiments for the lower initial concentration of cefuroxime using a reduced half-life (*Figure 4, CXM 2:r*).

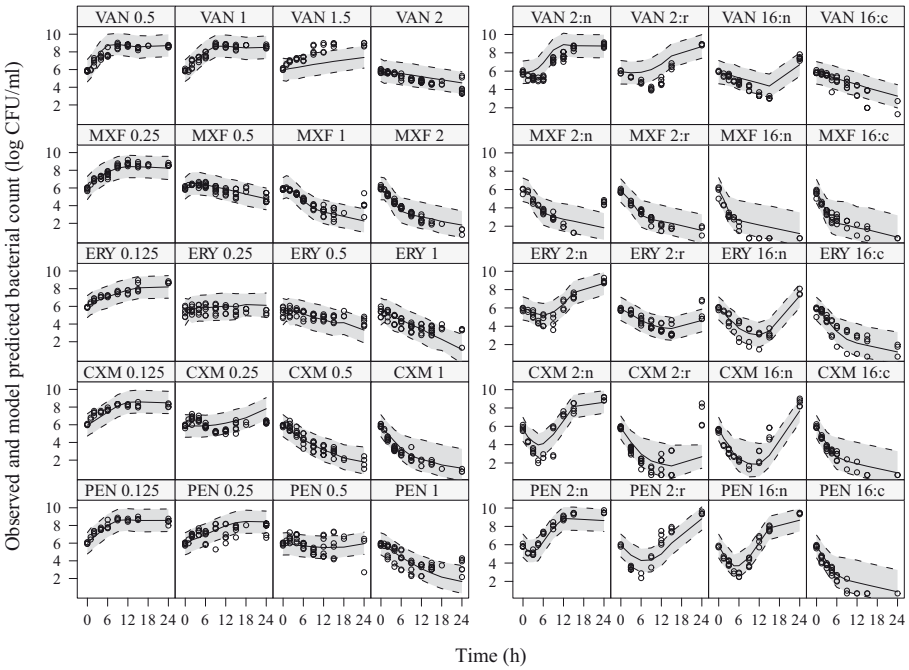

Figure 4. VPCs for the final PKPD model describing the growth and killing kinetics of an *S. pyogenes* strain using estimation based on the combined dataset (static and dynamic experiments). The figure shows a selection of the static time-kill curves (left) and the dynamic time-kill curves (right) with observations (◦) and model predictions as medians (solid lines) and 95% PIs (broken lines). Observed bacterial counts below the LOD are plotted as 5 CFU/ml. PEN, benzylpenicillin; CXM, cefuroxime; ERY, erythromycin; MXF, moxifloxacin; VAN, vancomycin. The number in panel strip indicates the start concentration of the antibiotic as a multiple of the MIC; n, simulated human half-life; r, simulated 1/3 of human half-life; c, constant concentration in the *in vitro* kinetic system.

Final parameter estimates (typical value and relative standard error, RSE%) from separate and simultaneous analyses of data from the static and dynamic experiments are presented in Table 3. A sigmoidal E_{max} model gave a significantly better fit than the ordinary E_{max} model (γ set to 1) for all five antibio-

tics. For erythromycin, γ was estimated to be less than one, indicating a more shallow concentration-effect relationship than for the other studied drugs. In some situations, the sigmoidicity factor was estimated to very high values (>50). Because these high values could result in mathematical problems in the minimization process, γ was fixed at the lowest value that did not have an effect on the fit (in this case, 20). Implementation of a mixture model that allowed for variations in the fraction of bacteria in the resting stage at the start of the experiment significantly improved the fit (resulting in a drop in OFV of 279 units).

Table 3. *Parameter estimates (typical value and RSE%) of bacterium-specific and drug-specific parameters describing the effects of five antibacterial drugs on S. pyogenes, as estimated from separate or combined analyses of static and dynamic time-kill curve data. PEN, benzylpenicillin; CXM, cefuroxime; ERY, erythromycin; MXF, moxifloxacin; VAN, vancomycin. FIX, parameter not estimated.*

Parameter	**Type of data used in parameter estimation**		
	Static	**Dynamic**	**Static and dynamic**
k_{growth} (h^{-1})	1.35 (5.1)	1.61 (6.2)	1.46 (4.1)
k_{death} (h^{-1})	0.182 (6.8)	0.342 (12)	0.187 (6.2)
B_{max} ($CFUml^{-1}$)	4.13×10^{8} (8.4)	7.24×10^{8} (32)	5.00×10^{8} (8.7)
f_{pers} (-)	0.0547 (48)	0.00810 (54)	0.0652 (49)
f_{Mix1} (-)	0.747 (16)	0.698 (21)	0.880 (7.5)
E_{max} PEN (h^{-1})	2.44 (8.4)	3.08 (5.2)	2.70 (7.4)
E_{max} CXM (h^{-1})	3.32 (6.6)	2.73 (5.4)	2.72 (5.1)
E_{max} ERY (h^{-1})	2.03 (6.2)	3.24 (26)	2.46 (13)
E_{max} MXF (h^{-1})	3.21 (4.5)	3.34 (8.0)	3.40 (4.6)
E_{max} VAN (h^{-1})	1.36 (5.1)	1.64 (5.3)	1.52 (5.3)
EC_{50} PEN (mgL^{-1})	0.00436 (8.0)	0.00768 (8.9)	0.00531 (25)
EC_{50} CXM (mgL^{-1})	0.00832 (7.8)	0.0124 (15)	0.00787 (8.3)
EC_{50} ERY (mgL^{-1})	0.0275 (14)	0.0953 (119)	0.0336 (44)
EC_{50} MXF (mgL^{-1})	0.0745 (2.8)	0.145 (3.3)	0.0750 (6.9)
EC_{50} VAN (mgL^{-1})	0.384 (0.9)	0.298 (2.9)	0.304 (13)
γ PEN (-)	1.29 (11)	20 FIX	1.06 (24)
γ CXM (-)	1.69 (8.3)	20 FIX	1.35 (14)
γ ERY (-)	0.770 (19)	0.461 (21)	0.652 (20)
γ MXF (-)	1.59 (6.8)	20 FIX	1.42 (7.6)
γ VAN (-)	20 FIX	20 FIX	4.99 (24)
k_{e0} PEN (h^{-1})	0.990 (9.6)	100 FIX	100 FIX
k_{e0} CXM (h^{-1})	0.848 (19)	100 FIX	100 FIX
k_{e0} ERY (h^{-1})	100 FIX	100 FIX	1.02 (21)
k_{e0} MXF (h^{-1})	0.635 (20)	0.893 (4.5)	0.627 (18)
k_{e0} VAN (h^{-1})	100 FIX	100 FIX	100 FIX
ε (-)	0.986 (10)	1.44 (9.4)	1.40 (7.4)
ε_{repl} (-)	0.465 (4.7)	0.475 (5.2)	0.468 (3.7)

In general, there was good agreement between the parameter estimates based on static and dynamic time-kill curve experiments and the parameters were estimated with acceptable precision. However, due to the sparseness of the dynamic data, a separate analysis of these data resulted in greater uncertainty (RSE%) for most model parameters. There was also a trend for lower values of γ when using the combined dataset, which could have been a result of differences between the static and dynamic data and/or increased information content in the combined dataset. An additive residual error model using ln-transformed data adequately described the random variability. The replicate residual error (ε_{repl}) and overall residual error (ε) was estimated to 0.47 and 1.40, respectively, using the combined analysis, corresponding to 0.20 and 0.61, respectively, using $\log_{10}$-transformed data.

Adaptive resistance of gentamicin (Paper IV)

A third set of static and dynamic time-kill curve experiments was performed in order to characterize the bactericidal activity of gentamicin on a strain of *E. coli* (*Figure 5*). Initially, exposure to gentamicin caused a rapid and extensive killing of the bacteria. However, this was followed by bacterial regrowth in the majority of the experiments. The presence of adaptive resistance was evident from both the static and dynamic time-kill curve experiments and the bacterial killing was consistently limited with subsequent doses for experiments using repeated dosing.

The general semi-mechanistic PKPD model (Papers I–II) was applied to the data and the model was further developed to include the development and reversal of adaptive resistance. The final model (*Figure 2*) characterized the development of adaptive resistance by a binding function and, as indicated by the VPCs (*Figure 5*), it described the observed data well for both static and dynamic experiments. Alternative models (e.g. those with a time- and concentration-dependent EC_{50} or a bacterial compartment with adaptive resistant bacteria) were tested, but did not fit the data as well.

Parameter estimates (typical value and 95% CI) are presented in Table 4. The antibacterial effect was adequately described by a simple E_{max} model (γ set to 1). k_{SR} was set to zero at low bacterial counts to describe the pronounced bacterial killing observed following high gentamicin exposures, and the transfer from the sensitive stage into the resting stage only occurred when the total bacterial content in the system exceeded an estimated breakpoint (2.09×10^6 CFU/ml). The rate constant for the development of adaptive resistance in the presence of gentamicin (k_{on}) was estimated at 0.0426 $Lmg^{-1}h^{-1}$. The rate constant for the bacteria to return to the susceptible state after developing adaptive resistance (k_{off}) was, because of insufficient information in the data, fixed at 50 hrs. The implementation of a mixture model to account for variations in the start inoculum was not supported by the data.

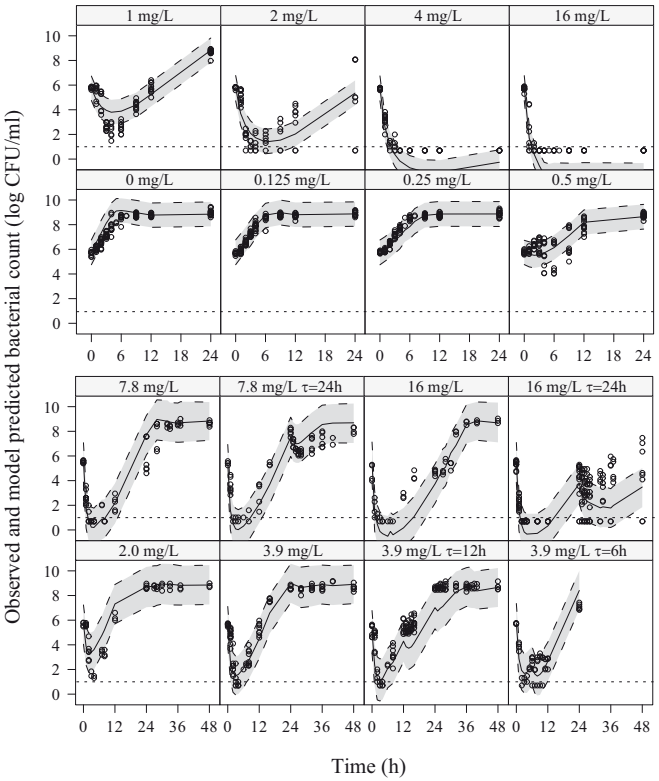

Figure 5. VPCs for the PKPD model describing the growth and killing kinetics of an *E. coli* strain after static (top) and dynamic (bottom) exposure to gentamicin. Shown are observed bacterial counts (◦) and model predictions as medians (solid lines) and 80% PIs (broken lines) of simulated data. The LOD (10 CFU/ml) is included as a dotted line; observed bacterial counts below the LOD are plotted as 5 CFU/ml.

Table 4. *Parameter estimates (typical value and 95% CI obtained from LLP) for the PKPD model describing the killing of E. coli by gentamicin. FIX, fixed parameter.*

Parameter	**Typical value**	**95% CI**
k_{growth} (h^{-1})	2.00	1.89 – 2.27
k_{death} (h^{-1})	0.179 FIX	-
B_{max} ($CFUml^{-1}$)	8.26×10^{8}	6.18×10^{8} – 11.1×10^{8}
Breakpoint ($CFUml^{-1}$)	2.09×10^{6}	1.01×10^{6} – 3.32×10^{6}
E_{max} (h^{-1})	51.0	44.6 – 61.6
EC_{50} (mgL^{-1})	9.93	8.45 – 12.1
AR_{50} (-)	0.113	0.0983 – 0.146
k_{on} ($Lmg^{-1}h^{-1}$)	0.0426	0.0376 – 0.0478
k_{off} (h^{-1})	0.0139 FIX	-
ε_{static} (-)	1.69	1.52 – 1.83
$\varepsilon_{dynamic}$ (-)	2.80	2.56 – 2.98
ε_{repl} (-)	0.618	0.590 – 0.649

Evaluation of model performance

Internal model evaluation (Papers I–II, IV)

The VPCs (*Figure 4–5*) showed that the predictions from the PKPD models were in good agreement with the original data in Papers I–II and IV. The model that was developed using experiments with static drug concentrations was also evaluated using internal validation with XV and CDD (Paper I). When the XV was used, the model showed good internal predictability. CDD indicated that the model resulted in robust parameter estimates, as only two parameters (f_{pers} and k_{e0}) changed substantially (>20%) when parts of the data were excluded. The sensitivity of f_{pers} to data exclusion was expected, since this has a direct influence on the fraction of experiments in the different subpopulations. The sensitivity of k_{e0} indicates that not all experiments supported the estimation of the time-delay.

Model performance across experimental design (Paper II)

The performance of the developed PKPD model in predicting observed bacterial counts across experimental designs was assessed using simulation-based diagnostics. The model developed in paper I (static data alone) was applied to the dynamic dataset without parameter re-estimation, i.e using the dynamic data as an external evaluation dataset. For the majority of the dynamic time-kill curve experiments, the observed bacterial counts were reasonably well predicted by the PKPD model with parameter estimates based only on the static data (*Figure 6*). However, for vancomycin, the killing effect in the dynamic experiments with the lower initial concentration (2×MIC) persisted a longer time period than anticipated by the analyses of the static experiment alone. This prolonged killing effect was not observed when the bacteria were exposed to a higher initial concentration (16×MIC). In contrast to these findings, for cefuroxime a faster regrowth was observed in the dynamic experiments than predicted by the static experiments alone.

A covariate analysis was carried out to search for general differences between the static and dynamic experiments, using experimental setup as a categorical covariate. When separate parameter estimates based on the type of experiment were allowed, a higher k_{growth} (1.8 versus 1.3 h^{-1}) and a higher k_{death} (0.41 versus 0.17 h^{-1}) were found for the dynamic experiments. No general trend was found for the drug-specific parameters; however, for three of the five studied drugs, a statistically significantly higher E_{max} (benzylpenicillin, erythromycin, and vancomycin) and/or a higher γ (benzylpenicillin, cefuroxime, and moxifloxacin) were found for the dynamic experiments. There were no differences in growth characteristics between growth control experiments performed in test tubes (i.e. static setting) and in the *in vitro* kinetic system (i.e. dynamic setting).

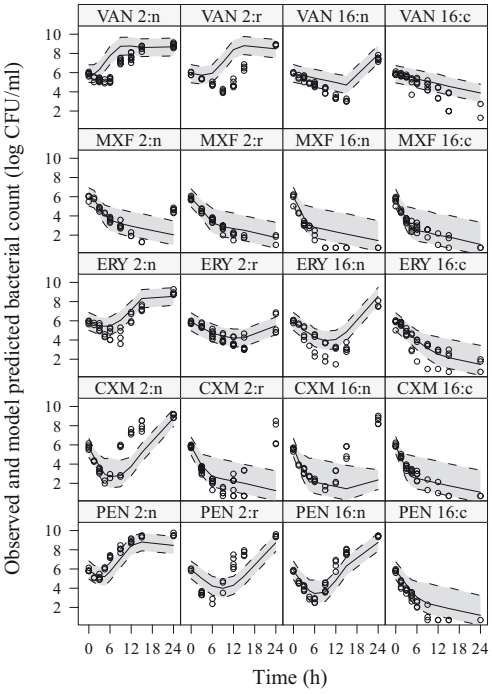

Figure 6. Observed time-kill curves for the dynamic experiments with model predictions (as medians and 95% PI) using parameter estimates based on data from static experiments only (external validation). Observed bacterial counts below the LOD are plotted as 5 CFU/ml. PEN, benzylpenicillin; CXM, cefuroxime; ERY, erythromycin; MXF, moxifloxacin; VAN, vancomycin. The number in the panel strip indicates the start concentration of the antibiotic as a multiple of the MIC; n, simulated human half-life; r, simulated 1/3 of human half-life; c, constant concentration in the *in vitro* kinetic system.

Population PK of gentamicin in newborn infants

Patients and collected data

Sixty-one preterm and term newborn infants (GA 23–42 weeks, PNA 0–45 days) were enrolled in the study. The median gentamicin treatment duration was 6 days (range 2–19 days) and, besides the study treatment course, 17 of the neonates received 1–3 additional courses of gentamicin treatment. In total, 894 serum gentamicin concentrations were available for the PK analysis, with a median of 12 samples per individual (*Figure 7*). Twenty-five percent of the measured concentrations were "peak" samples (collected 0.5–1.5 hrs after the end of the infusion) and 29% were "trough" samples (collected 18–25 hrs after the end of the infusion).

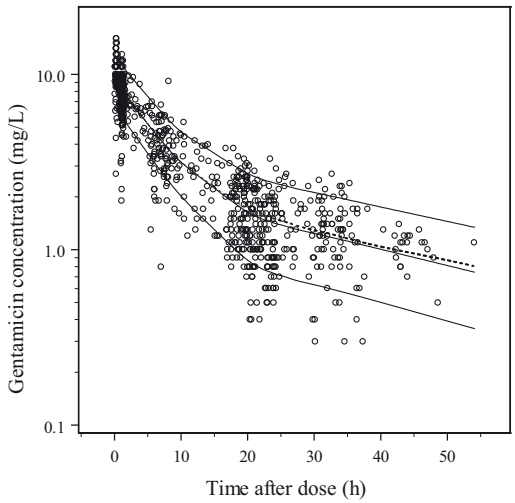

Figure 7. Observed gentamicin concentrations (◦), loess smooth of observed data (dashed line) and loess smooth of simulated data as median and 90% PIs (solid lines) versus time after dose.

Population PK model

The basic PK model was a 3-compartment model. A combined additive and proportional residual error model was applied to ln-transformed data with IIV included in the CL, the central volume of distribution (V_1) and the residual error magnitude. When parameter dependence on WT according to allometric scaling was included, there was a pronounced drop in OFV (of 350 units) and reductions in IIV for CL (from 81% to 37%) and V_1 (from 81% to 13%). All three age characteristics (i.e. PNA, GA, PMA) were identified as having a significant influence on CL. However, the most influential age variable was PNA and, in the final model, CL was dependent on both PNA

and GA, which were included according to a non-linear power function and a linear function, respectively. Including these age-dependent variables for CL resulted in a total drop in OFV of 475 units and a decrease in IIV from 37% to 22%. GA was also identified as having a significant influence on V_1 with preterm neonates having a slightly larger V_1 in L/kg than term neonates (drop in OFV of 14 units). Using these data, neither serum creatinine nor serum cystatin C were significant covariates for gentamicin CL. The parameter estimates for the final model are summarized in Table 5.

Model evaluation

NPC stratified for term and preterm (GA <37 weeks) neonates showed the 90% PI to include 88.4% of the data for the term neonates and 91.8% of the data for preterm neonates. Further, when applying the final model without re-estimation to the evaluation dataset (external validation), the population predicted concentrations were unbiased, with an MPE of -3.6% and the precision (estimated by MAPE) was 28.4%. The bias and precision were 2.9% and 28.1%, respectively, for term neonates and -6.7% and 28.5%, respectively, for preterm neonates. The structural parameter estimates were also confirmed to be similar (within 10%) for the original and a combined (original + external) dataset.

Table 5. *Population parameter estimates (typical value and 90% CI obtained from a non-parametric bootstrap) for the final PK model.*

Parameter	**Typical value (90% CI)**
CL ($Lh^{-1}kg^{-3/4}$)[a]	0.00999 (0.00784–0.0127)
θ_{GACL} ($week^{-1}$)[a]	0.0862 (0.0729–0.0994)
θ_{PNACL}(-)[a]	0.548 (0.468–0.638)
V_1 (Lkg^{-1})[b]	0.405 (0.388–0.421)
θ_{GAV1} ($week^{-1}$)[b]	-0.0116 (-0.0173– -0.0058)
Q_2 ($Lh^{-1}kg^{-3/4}$)	0.0939 (0.0793–0.111)
V_2 (Lkg^{-1})	0.310 (0.282–0.341)
Q_3 ($Lh^{-1}kg^{-3/4}$)	0.0173 (0.0108–0.0225)
V_3 (Lkg^{-1})	5.07 (2.49–7.52)
ε_{Add} (mgL^{-1})	0.135 (0.088–0.178)
ε_{Prop} (%)	15.2 (13.2–17.4)
IIV CL (%)	21.6 (15.0–27.1)
IIV ε (%)	43.0 (31.5–54.5)

[a] $TVCL = CL \times WT^{3/4} \times (1 + \theta_{GACL} \times (GA - GA_{median})) \times (1 + PNA^{\theta_{PNACL}})$, GA_{median}=28.9 weeks

[b] $TVV_1 = V_1 \times WT \times (1 + \theta_{GAV1} \times (GA - GA_{median}))$, GA_{median}=28.9 weeks

Gentamicin dosing schedules in newborn infants

Based on simulations from the final PK model (Paper III) using the covariate values for the study neonates, the majority of the term neonates receiving the standard dosing regimen of 4 mg/kg given once daily met the traditionally used therapeutic target concentrations, with peak concentrations >8 mg/L (1 hr post-infusion) and trough concentrations <2 mg/L (*Figure 8*). However, following the same dosing regimen, peak gentamicin concentrations were low (<8 mg/L) for preterm neonates in the first and often critical days of therapy (*Figure 8*, *top*). For the extremely preterm neonates (GA <28 weeks), the median trough gentamicin concentrations were also predicted to be consistently >2 mg/L, indicating an increased risk of nephrotoxicity. In order to reach the traditionally therapeutic targets higher loading doses (4.5–5 mg/kg) and longer dosing intervals (36–48 hrs) would thus be needed for the preterm newborn infants.

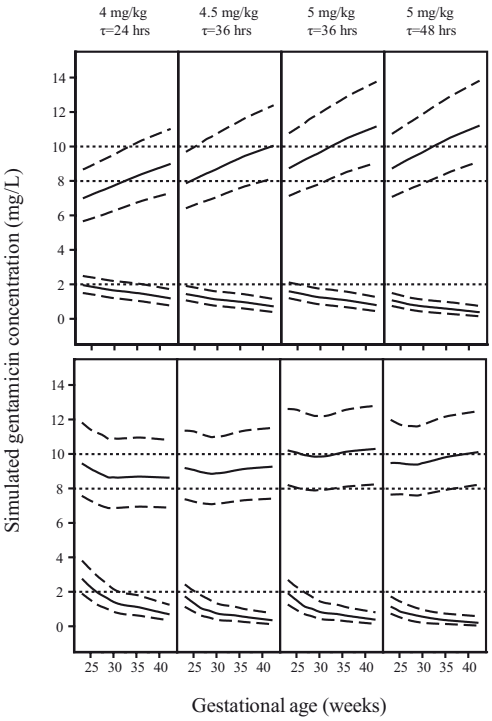

Figure 8. Simulated trough and peak (1 hr post-infusion) gentamicin concentrations as medians (solid lines) and 10th and 90th percentiles (dashed lines) versus GA after the first gentamicin dose (top) and after one week of treatment (bottom) for newborn infants following four dosing regimens.

Predictions for 4 newborn infants (GA 25, 29, 34 and 40 weeks) were performed based on the typical parameter estimates from the final PK model in Paper III and the PKPD model developed from the time-kill curves in Paper IV. Although preterm neonates reaches lower peak gentamicin concentrations (<8mg/L), the overall bacterial killing was predicted to be higher than that for the term neonates (*Figure 9*). When comparing the standard dosing regimen of 4 mg/kg every 24 hrs with the alternative regimens of 4.5 mg/kg every 36 hrs or 5 mg/kg every 48 hrs, the bacterial counts was predicted to have reached the levels used as the starting inocula (5×10^5 CFU/ml) earlier following the extended dosing interval regimens (*Figure 9*). Unless the difference in the initial killing phase is of high importance, doses higher than 4.5 to 5 mg/kg are thus indicated in order for the extended dosing interval regimens to be as effective as once-daily dosing. When the dose was increased (4–7 mg/kg), only minor differences in bacterial killing were observed initially (first 6 hrs post-dose), however, by the time of the second dose, the predicted difference in bacterial counts between the highest and lowest dose was several $\log_{10}$s (*Figure 10*). No major advantage was observed for a fractionated schedule over 36 hrs when a single dose of 6 mg/kg was compared with fractionated schedules with the same total dose.

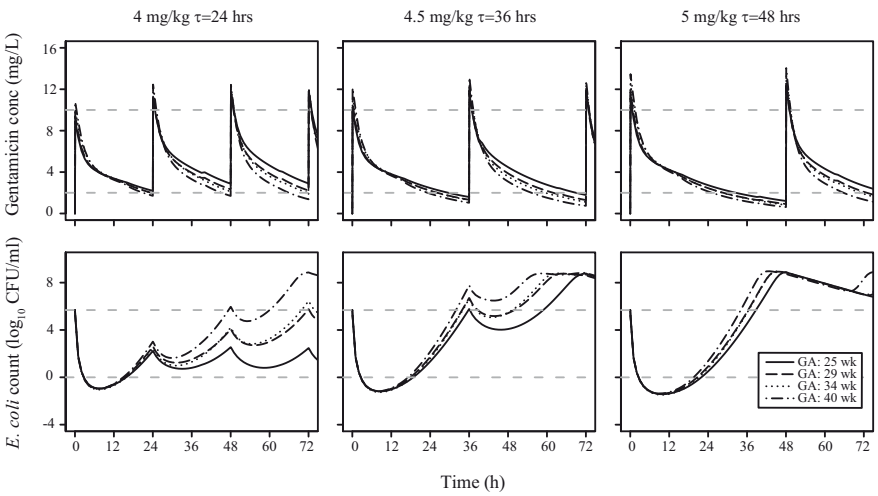

Figure 9. Model predictions of gentamicin concentrations (top) and *E.coli* counts (bottom) for four typical newborn infants with varying GA following three commonly used dosing regimens. Grey dashed lines represent gentamicin concentrations of 2 and 10 mg/L (top) or bacterial counts of 5×10^5 and 1 CFU/ml (bottom).

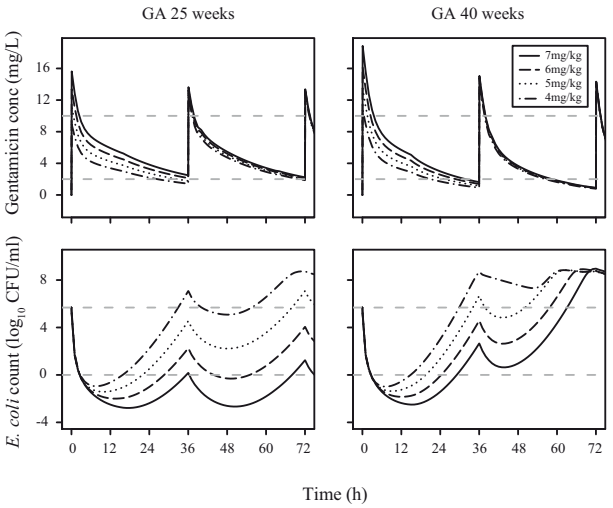

Figure 10. Model predictions of gentamicin concentrations (top) and *E.coli* counts (bottom) for different initial doses (4–7 mg/kg) and subsequent doses of 5 mg/kg every 36 hrs. Predictions are for a preterm neonate (GA 25 weeks, left) and a term neonate (GA 40 weeks, right). Grey dashed lines represent gentamicin concentrations of 2 and 10 mg/L (top) or bacterial counts of 5×10^5 and 1 CFU/ml (bottom).

Model performance in predicting PK/PD indices

Default simulation setting

The developed PKPD models (Papers I–II, IV) were evaluated for their capacity to predict the commonly used PK/PD indices for the six drugs investigated in the thesis. A dose-fractionation study was simulated for each drug and the relationship between the model-predicted PD endpoint (i.e. bacterial count after 24 hrs of treatment) and the three PK/PD indices are shown in *Figure 11*. The coefficients of determination and the magnitude of the most correlated PK/PD index (as determined in the default simulation setting) required for a bacteriostatic and bactericidal effect, respectively, are presented in Table 6.

For the ß-lactam antibiotics (benzylpenicillin and cefuroxime), $fT_{>MIC}$ was selected to be the PK/PD index most predictive of the PD endpoint (*Figure 11*). For these drugs there was also a clear benefit associated with dose-fractionation schedules, with lower doses administered more frequently. For erythromycin, gentamicin, moxifloxacin and vancomycin, the *f*AUC/MIC was selected as the PK/PD index showing the highest correlation with the antibacterial effect, and the effects of these drugs were less dependent on the dosing frequency (*Figure 11*).

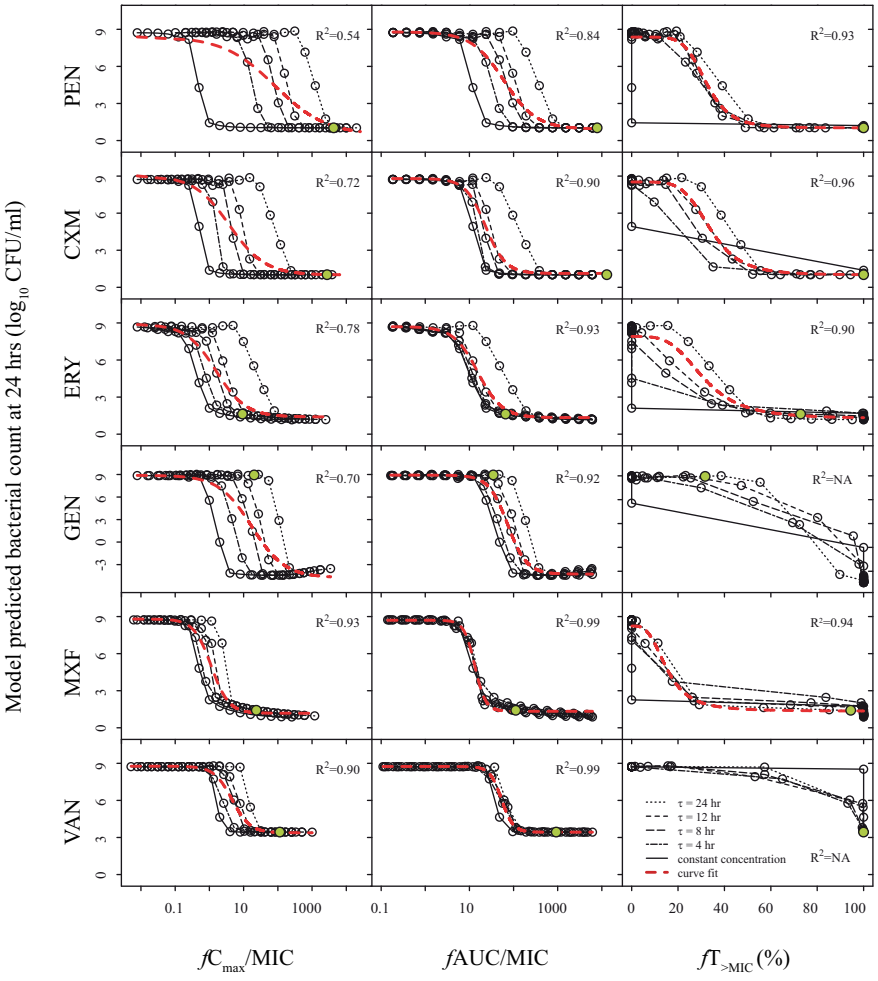

Figure 11. Relationship between the model-predicted bacterial count after 24 hrs of therapy and the three PK/PD indices after treatment with benzylpenicillin (PEN), cefuroxime (CXM), erythromycin (ERY), gentamicin (GEN), moxifloxacin (MXF), or vancomycin (VAN). Each simulated dosing regimen (i.e. dose and dosing interval) are represented by one data point in each panel. The non-linear least square regression line (red dashed line) and the coefficient of determination (R^2) are included. The predictions for clinically commonly used dosing regimens are indicated in green. NA, not estimable.

The clinically commonly used dosing regimens were predicted to achieve a close to maximum effect for all drugs except for gentamicin (*Figure 11*). For gentamicin, standard dosing regimens of 4.5–7 mg/kg given once daily were here predicted to result in a fast initial killing phase followed by bacterial regrowth at 24 hrs.

Altered simulation setting

The consistency of the PK/PD indices across different study conditions was investigated and a summary of the results are presented in Table 6.

- *Modification of PK.* When CL was reduced to one third of its original value or by simulating the PK profile of a preterm neonate (benzylpenicillin and gentamicin), both fC_{max}/MIC and *f*AUC/MIC showed higher correlations with the PD endpoint for all investigated drugs (Table 6). With this simulation setting, *f*AUC/MIC was at least as good a predictor of the effect as $fT_{>MIC}$ for the ß-lactam antibiotics. Reducing the CL also had an impact on the magnitude of the PK/PD index needed to achieve a bacteriostatic or bactericidal effect. For the β-lactams, a shorter $fT_{>MIC}$ was needed to achieve the same effect. Similarly, a reduced *f*AUC/MIC was needed to reach the same antibacterial effect for erythromycin and gentamicin (Table 6).
- *Uncertainty in MIC.* The MIC was highly influential on the correlation between $fT_{>MIC}$ and the PD endpoint, and a change in the MIC influenced both the selection and the magnitude of the PK/PD index (Table 6). As expected, the change in MIC did not influence the correlations for fC_{max}/MIC or for *f*AUC/MIC when the PK was unchanged, and the magnitude of these PK/PD indices needed for effect was changed in proportion to the MIC.
- *Alternative PD endpoints.* In general, the choice of PD endpoint did not have a large impact on the selection or on the magnitude of the PK/PD index.
- *Modification of the concentration driving the antibacterial effect.* When the antimicrobial killing effect was driven by the antibiotic concentration in one of the peripheral compartments, both fC_{max}/MIC and *f*AUC/MIC showed higher correlations with the PD endpoint. Similarities can be found with the simulations when CL was reduced since both alterations will result in reduced fluctuations in the concentration-time profile.

Table 6. Coefficients of determination (R^2) and magnitudes of $fT_{>MIC}$ (benzylpenicillin and cefuroxime) or *f*AUC/MIC (erythromycin, gentamicin, moxifloxacin and vancomycin) required for a bacteriostatic (B_{STAT}) or bactericidal (B_{CID}) effect.

Setting	R^2			B_{STAT}	B_{CID}
	fC_{max}/MIC	*f*AUC/MIC	$fT_{>MIC}$		
Benzylpenicillin					
Default	0.54	0.84	0.93	29	38
Reduced CL	0.73	0.94	0.93	25	33
PK neonate	0.86	0.98	0.91	24	32
0.5×MIC	0.54	0.84	0.98	40	52
2×MIC	0.54	0.84	0.88	20	26
Cefuroxime					
Default	0.72	0.90	0.96	30	41
Reduced CL	0.89	0.99	0.98	17	21
0.5×MIC	0.72	0.90	0.98	45	56
2×MIC	0.72	0.90	0.87	15	31
Erythromycin					
Default	0.78	0.93	0.90	11	34
Reduced CL	0.93	1.0	0.91	8.5	19
0.5×MIC	0.78	0.93	0.96	22	67
2×MIC	0.78	0.93	0.80	5.5	17
Gentamicin					
Default	0.70	0.92	NA	39	67
Reduced CL	0.86	0.98	NA	28	42
PK neonate	0.91	0.99	NA	26	37
0.5×MIC	0.71	0.92	NA	79	133
2×MIC	0.71	0.92	0.94	20	33
Moxifloxacin					
Default	0.93	0.99	0.94	11	19
Reduced CL	0.97	0.99	NA	11	19
0.5×MIC	0.93	0.99	0.98	23	37
2×MIC	0.93	0.99	0.85	5.7	9.4
Vancomycin					
Default	0.90	0.99	NA	54	NA
Reduced CL	0.93	0.99	NA	51	NA
0.5×MIC	0.90	0.99	NA	109	NA
2×MIC	0.90	0.99	NA	27	NA

NA, not estimable

Discussion

Semi-mechanistic PKPD model

This thesis describes the development of a novel semi-mechanistic PKPD model that accurately predicts the time course of events seen in a bacterial culture exposed to antibacterial drugs of different classes. Despite its fairly simple structure, the PKPD model showed good predictive performance for a wide range of both static and dynamic antibacterial drug exposures. It was also found that the model could be applied to another bacterial strain and drug combination and that the semi-mechanistic structure allowed for additional complexity such as the adaptive resistance of gentamicin.

In Papers I and II, we exposed the same bacterial strain to five antibiotics of different classes. All data were analyzed simultaneously in order to separate bacterium-specific parameters describing the kinetic behavior of the bacterial system from drug-specific parameters describing the imposed drug effect. This is, to our knowledge, the first time that a PKPD model has been simultaneously fitted to data from several antibacterial drugs of different classes. This model makes it easy to compare the antibacterial effects of different drugs and because of the mechanistic structure of the model and that the model already includes information regarding the underlying bacterial system, new agents can be evaluated and compared by carrying out only a limited number of additional experiments.

The developed PKPD model consists of two bacterial stages representing normally growing drug-susceptible cells and resting drug-insusceptible cells. This structure was used to account for the biphasic killing behavior seen in the time-kill curve experiments in Paper I, as well as the reduced growth rate and reduced drug-sensitivity at high bacterial levels. The structure resembles phenotypic switching between normally growing cells and persister cells with reduced growth rates and reduced antibiotic susceptibility [43-44]. The model developed here has structural similarities to the model proposed by Yano et al. [142-143]. Their model also describes two bacterial stages representing drug-susceptible and drug-insusceptible cells, respectively. However, that model does not include the transition of growing cells into persister cells when high bacterial levels are reached in the system, i.e. when approaching the stationary phase. Thus, their model is not able to characterize the reduction in drug susceptibility at high bacterial levels.

Except for a few experiments with moxifloxacin (1×MIC), the time-kill curve data used in the initial model development (Papers I–II) did not indicate any presence or emergence of resistance and the PKPD model was therefore not developed to capture such phenomena. The semi-mechanistic structure of the model does allow, however, for adding additional complexity and, when the model was applied to the gentamicin data in Paper IV, a binding model with two compartments representing the degree of resistance development was included to describe the emergence of adaptive resistance to gentamicin. In this model, gentamicin triggers the development of adaptive resistance, which results in a graded reduction in the E_{max} value and thereby a reduced bacterial killing. The model also allows the resistance to be reversible, although there was limited information in these *in vitro* experiments to estimate the return to the drug-susceptible stage, which limits the use of the model in making predictions beyond the studied time period.

Another approach to describing the change in killing behavior over time has been to include a concentration- and/or time-dependent adaptation factor influencing either k_{growth} [52] or EC_{50} [53-55]. This is, however, a rather empirical approach which, based on our experimental data, did not satisfactorily capture the biphasic killing behavior seen in Paper I or the adaptive resistance seen in Paper IV. It has also been suggested that a change in the killing rate over time could be explained by heterogeneity of the bacterial population, with a number of subpopulations present in the start inoculum with different susceptibilities to drug treatment [52, 56, 58-59]. In those earlier studies, the heterogeneous bacterial populations were generally observed in rather high start inocula (10^7–10^8 CFU/ml). In our experiments, a lower start inoculum (10^5–10^6 CFU/ml) was used and the presence or development of true genetic resistance was thought not to be the explanation for the decrease in the killing rate.

Initially, in Paper I, the model was developed from static time-kill curve experiments only. These experiments are rather simple in nature and therefore allow a wide range of concentrations to be studied and the full concentration-effect relationship to be explored. Another advantage of static experiments is that they allow for easier detection of the presence of persister cells than is possible with experiments in a kinetic system, where bacterial regrowth due to a declining drug concentration can mask such phenomena. However, for the PKPD model to be useful in predicting clinical effects, which is most often the overall aim, it is important that it is able to capture the effects seen following concentration-time profiles that mimic those observed *in vivo*. Therefore, a second set of experiments in Paper II was used to confirm that the structure of the PKPD model was applicable across experimental designs and that the model was able to accurately capture the time course of antibacterial effects seen in bacterial cultures when the concentration of the antibiotic decreased over time, with similar half-lives to those observed in patients. It was also shown that the experiments with static con-

centrations were in most cases informative in predicting the time-kill curves following dynamic concentration-time profiles. This could limit the need for the more time and labor consuming dynamic experiments, as the antibacterial effect following dynamic concentration exposures can be simulated by including the PK model describing the change in drug concentration over time.

Most data from static and dynamic time-kill curve experiments have previously been modeled separately. In only a couple of recent examples have data from static and dynamic experiments been simultaneously analyzed but, in these examples, common parameter estimates were generally used for the two experimental settings [41, 144]. In our study, we evaluated potential differences between the two *in vitro* systems (i.e. static and dynamic) using a modeling approach. There were no significant differences between the two experimental settings in the growth kinetics of the control experiments (i.e. without antibiotics). While significant differences in drug-specific parameter estimates were found when all the data were used in the estimation, there appeared to be no general trend that was consistent for all the studied drugs. For vancomycin, the sustained suppression of bacterial growth observed in some dynamic experiments (2×MIC as initial concentrations with simulated normal and reduced half-lives) was not well predicted by the model. This is, however, in line with previous findings of differences in the antibacterial effects between exposure to sub-MICs alone (SME) and to sub-MICs following concentrations exceeding the MIC (PAE-SME) [11, 145]. The presence of a long PAE-SME compared to the SME alone could indicate that a more extensive PD characterization is required than that provided by the static drug exposures and the developed model. In contrast to the results for vancomycin, for bacteria exposed to cefuroxime the regrowth was faster in the dynamic experiments than that predicted from the parameter estimates from the static experiments alone. However, the predictions improved significantly when both static and dynamic type data were used in the parameter estimation, without a negative impact on the model fit of the static time-kill curve experiments.

Gentamicin treatment in newborn infants

Gentamicin PK has previously been described using 3-compartment models in adults, with slow redistribution from the tissue compartment and terminal half-lives ranging from 30 to 700 hrs [62-63]. In Paper III, the disposition of gentamicin in neonates was characterized by a 3-compartment PK model with half-lives for the three phases of 1.3, 15 and 425 hrs, respectively, for the typical neonate in the study population. The model predicted that a term neonate (GA 40 weeks) would have a CL of 0.019 L/h/kg$^{3/4}$ at birth, whereas a extremely preterm neonate (GA 24 weeks) would have a CL as low as

0.0057 L/h/kg$^{3/4}$ at birth. By one week later, the model predicted that CL would have increased 3.7-fold in both cases. After correcting for differences in covariate distribution in the study populations, this is in line with what has been reported previously [110-111, 115].

A correlation between gentamicin CL and either GA [112, 115, 146] or PNA [109, 111] has been reported in previous studies in the neonates. In our Paper III study, the majority of the neonates started gentamicin treatment on the first or second day of life and as many as 35% of the samples were taken during the first four days of life, i.e. when the GFR is known to change rapidly. The prospective study design allowed for additional sampling at time-points other than those used for routine TDM and also ensured data of high quality. Because of the relatively frequent sampling per individual and the wide range of GAs, it was possible to characterize the developmental PK of gentamicin with respect to both GA and PNA. It has been suggested that maturational changes with respect to age could best be characterized using the PMA (i.e. GA + PNA) [89]. However, separating the influence of GA and PNA has the advantage of being able to also capture the rapid increase in GFR that occurs during the first days after birth and we regard it to be of high importance to accurately describe the PK in these often critical days of therapy.

Neither serum creatinine nor serum cystatin C were found to be predictive markers for gentamicin CL. Measurements of the marker substance should be made under steady-state conditions between production and elimination in order to reflect renal function. An assessment of renal function using endogenous markers is therefore difficult during the first period of life. Not much is known about the production rate in this period, but there is a known rapid change in elimination capacity and usually also a highly variable hydration status [67]. Serum creatinine levels measured soon after birth are also known to reflect maternal levels as a result of placental transfer [83]. A couple of previous studies have shown gentamicin CL in neonates to be correlated with creatinine concentrations [110-112]. However, in those study populations, the median PNA was somewhat higher (>5 days) than in ours. Although cystatin C has been reported not to pass the placental barrier, not much is known about how cystatin C levels are affected by different pathological changes.

The effect target for gentamicin (fC_{max}/MIC >8–10 mg/L) is based on clinical studies in adult patient populations using multiple daily dosing schedules [32, 94]. In neonates, the treatment is often empirical and the MIC unknown. The therapeutic target currently in use is generally to achieve peak concentrations of >8–10 mg/L (1 hr post-infusion) and trough concentrations of <1–2 mg/L [68, 116]. According to PK model predictions, the majority of term neonates receiving the standard dosage of 4 mg/kg once daily would achieve these therapeutic target concentrations. However, following the same dosing regimen, preterm neonates are predicted to have low initial

gentamicin concentrations and trough concentrations consistently above 2 mg/L. This suggests the use of higher loading doses (4.5–5 mg/kg) and longer dosing interval (36–48 hrs). On the other hand, prolonging the dosing interval could allow surviving bacteria to re-grow between doses. Differences in the frequency with which the peak concentrations are achieved will probably also have an effect on the treatment outcome. In this situation it could, therefore, be of value to develop the dosing regimens from predictions based on the PK as well as a PKPD model characterizing the time course of the growth and killing kinetics of the bacteria.

In Paper IV, it was shown that, even though the peak concentrations were lower for preterm neonates, the overall higher drug exposure resulted in more pronounced bacterial killing compared to that in term neonates. It was also shown that, when extending the dosing interval to 36 or 48 hrs, higher peak concentrations than >8–10 mg/L were necessary in order for these schedules to be as effective as the once-daily dosing regimen. This suggests that the therapeutic target concentrations currently in use need to be re-evaluated. For all patients, the use of the aminoglycosides is limited by their ability to cause toxicity and a more detailed characterization of the concentration-toxicity relationship is also warranted in the design of optimal dosing regimens. Other limitations with these predictions are that they do not take the effect of the immune system into account (although maybe of less importance here since the neonates is regarded to be immunocompromised) and that the evaluations are performed without including inter-individual variability in PK and PD.

Role of PKPD in optimizing antibiotic dosage

In Paper V, it was shown that the PKPD models based on *in vitro* time-kill curve experiments (Papers I–II, IV) could well identify the commonly used PK/PD indices and the magnitude of these indices required for an *in vivo* effect, for all six antibacterial agents evaluated in the thesis. In accordance with previous findings in numerous *in vitro* and animal studies, $fT_{>MIC}$ was the PK/PD index that was best correlated with the PD endpoint for the two investigated ß-lactam antibiotics [22-26, 147], while *f*AUC/MIC was the best predictor of the effect for the remaining four antibacterial drugs (erythromycin, gentamicin, moxifloxacin and vancomycin) [25-27, 148].

Further, the choice and magnitude of the PK/PD index were shown to be sensitive to study design and the concentration-time profile (PK) of the drug in the studied patient population. The ß-lactam antibiotics had the shortest half-life, and were also the drugs where $fT_{>MIC}$ was the major predictor of effect. When the rate of elimination of these drugs was decreased to mimic patient groups with lower clearances, *f*AUC/MIC was at least as important as a predictor of the effect as $fT_{>MIC}$. Further, when long dosing intervals (i.e.

12 and 24 hrs) were excluded, *f*AUC/MIC became the PK/PD index that was most predictive of the effect for all evaluated drugs, which also is in line with previous reports [28]. The correlation between the $fT_{>MIC}$ and the effect was also highly dependent on the MIC and, since the MIC is generally measured with poor precision, this could be influential in the selection and magnitude of the PK/PD index. The MIC has several shortcomings and, since all PK/PD indices are based on the MIC, these shortcomings are carried over into the PK/PD indices.

After establishment of the magnitude of the PK/PD indices required for effect, these targets are commonly used in Monte Carlo simulations to support dosing regimens [35, 37]. In these simulations, variability in PK and the MIC are taken into account. However, the PK/PD targets are generally assumed to be independent of differences in PK and to remain consistent within a drug class and among species, pathogens, dosing regimens, and patient subpopulations, as long as variability in MIC and protein binding is accounted for. The simulations presented in Paper V clearly demonstrate that this is not always the case.

In the process of creating summary endpoints, such as the PK/PD indices, there is a loss of information which could limit their applicability across study conditions and study populations. A more advantageous approach in the design of dosing regimens would be to perform the Monte Carlo simulations on models that provide a full quantitative description of the time course of all simultaneously occurring processes (e.g. PK, effect, resistance) as is the case for the pharmacometric models developed in this thesis. Categorizations necessary for practical dosage recommendations to different subpopulations and bacterial susceptibilities (MICs) should be performed at the very end of the dose selection process, thus minimizing the loss of information and the use of potentially erroneous extrapolations related to summary endpoints such as the PK/PD indices.

Conclusions

In this thesis, pharmacometric models for antibacterial agents have been developed and evaluated and it has been illustrated how such models can be implemented in the design of dosing regimens.

- A novel semi-mechanistic PKPD model was developed to characterize bacterial growth and killing kinetics as observed in *in vitro* time-kill curve experiments. The model structure accurately described the changes in a bacterial system after a wide range of static and dynamic exposures to five antibacterial agents of different classes. The model was also applicable to another bacterial strain and antibacterial agent, and additional model components could be added to the structure to also capture the development of adaptive resistance.

- Simulation-based diagnostics were used to illustrate that the majority of the time-kill curves with dynamic drug concentrations could be reasonably well predicted by the model with parameter estimates taken from experiments with static drug concentrations. The use of a modeling approach could therefore limit the need for time- and labor-intensive experiments involving dynamic drug exposure.

- The currently used PK/PD indices were successfully selected by predictions from the final PKPD model for all six drugs investigated in the thesis. These findings support that PKPD models based on *in vitro* data can be predictive of the antibacterial effects observed *in vivo*. The selection and magnitude of the PK/PD indices were sensitive to study design and were not always consistent between sub-populations with different PK. The PK/PD indices appear therefore to extrapolate poorly across patient sub-populations, which limits their value in the dosage selection process.

- A population PK model for gentamicin was successfully developed from a prospective study in term and preterm newborn infants. WT and age (GA and PNA) were found to be major factors contributing to variability in gentamicin CL. In contrast, cystatin C and serum creatinine levels were not correlated with gentamicin clearance and are therefore not likely to be predictive markers of renal function in this patient population.

- Simulations based on the PK model showed that most term neonates achieve the traditionally targeted peak and trough gentamicin concentrations after a standard dosage of 4 mg/kg given once daily. However, for preterm neonates, higher loading doses and prolonged dosing intervals are needed. When the bacterial killing kinetics and adaptive resistance were also taken into consideration, it was shown that even though lower peak gentamicin concentrations are reached in preterm neonates, more extensive overall bacterial killing takes place because of higher total drug exposure (AUC). The currently used therapeutic targets thus needs to be re-evaluated and this further highlights the advantages of using PKPD models in the dose selection process.

Future perspectives

The use of pharmacometric models in the design of dosing regimens has increased in the last two decades. However, within the antibiotic area, the use of PKPD models characterizing the whole time course of the effect is still limited. This study supports mechanism-based PKPD modeling based on *in vitro* time-kill curve studies as a flexible and powerful tool for describing the effects of antibacterial agents. These models can be used in experimental and clinical study design as well as in the development of dosing guidelines tailored to specific patient populations.

Time-kill curve experiments are commonly performed early in the characterization of antibacterial drug effects and the semi-mechanistic model developed here is likely to find applications in the early phases of drug development. As presented, the structure of the PKPD model can be easily applied to other bacterial strains and antibiotics, and additional components can be added when needed. The model can also be used as prior information in optimal design. By optimizing the type of experiment to be conducted, including the concentration-time profiles as well as the number of samples taken and the timing of sampling, the information gained in future experiments can be maximized with minimal cost. *In vitro* experiments are flexible, informative and have ethical advantages over animal studies. However, to reach their full potential, it is necessary to make these methods less labor intensive by standardization and automatiziation. Such methodological refinements would also allow for the effect to be evaluated beyond the relatively limited study period normally used in these experiments.

Although the semi-mechanistic model structure described here was found to have good predictive properties, it still represents a simplification of a much more complex biological system. The model characterizes the effects observed in a homogeneous bacterial population. Important factors for future considerations are to also include pre-existing heterogeneity in drug susceptibility as well as the possibility of spontaneous or drug-imposed changes in susceptibility (i.e. the development of drug resistance). Other factors not captured include the host defense mechanisms, the pathology of the infection, and the virulence of the pathogen. Also not addressed in this thesis is the exposure-toxicity relationship. When such characterization is available, it can be combined with the PK and PKPD model and utilized in an estimation of an overall optimal dosing strategy by maximizing the total expected drug utility.

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// Bettan

References

1. Armstrong GL, Conn LA, Pinner RW. Trends in infectious disease mortality in the United States during the 20th century. JAMA. 1999 Jan 6;281(1):61-6.
2. Cohen ML. Changing patterns of infectious disease. Nature. 2000 Aug 17;406(6797):762-7.
3. ECDC/EMEA. The bacterial challenge: time to react. A call to narrow the gap between multidrug-resistant bacteria in the EU and the development of new antibacterial agents. EMEA/576176/2009. Joint technical report. 2009.
4. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009 Jan 1;48(1):1-12.
5. IDSA. Bad bugs, no drugs. As antibiotic discovery stagnates... a public health crisis brews. Infectious diseases society of america. 2004.
6. WHO. Priority Medicines for Europe and the World. WHO/EDM/PAR/20047. 2004.
7. Bhattaram VA, Bonapace C, Chilukuri DM, Duan JZ, Garnett C, Gobburu JV, et al. Impact of pharmacometric reviews on new drug approval and labeling decisions--a survey of 31 new drug applications submitted between 2005 and 2006. Clin Pharmacol Ther. 2007 Feb;81(2):213-21.
8. Lalonde RL, Kowalski KG, Hutmacher MM, Ewy W, Nichols DJ, Milligan PA, et al. Model-based drug development. Clin Pharmacol Ther. 2007 Jul;82(1):21-32.
9. Peck CC, Cross JT. "Getting the dose right": facts, a blueprint, and encouragements. Clin Pharmacol Ther. 2007 Jul;82(1):12-4.
10. Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: a review. Scand J Infect Dis Suppl. 1990;74:63-70.
11. Odenholt I. Pharmacodynamic effects of subinhibitory antibiotic concentrations. Int J Antimicrob Agents. 2001 Jan;17(1):1-8.
12. Gloede J, Scheerans C, Derendorf H, Kloft C. In vitro pharmacodynamic models to determine the effect of antibacterial drugs. J Antimicrob Chemother. 2010 Feb;65(2):186-201.
13. Gerber AU, Brugger HP, Feller C, Stritzko T, Stalder B. Antibiotic therapy of infections due to Pseudomonas aeruginosa in normal and granulocytopenic mice: comparison of murine and human pharmacokinetics. J Infect Dis. 1986 Jan;153(1):90-7.
14. Andes D, Craig WA. Animal model pharmacokinetics and pharmacodynamics: a critical review. Int J Antimicrob Agents. 2002 Apr;19(4):261-8.
15. Lowdin E, Odenholt I, Bengtsson S, Cars O. Pharmacodynamic effects of sub-MICs of benzylpenicillin against Streptococcus pyogenes in a newly developed in vitro kinetic model. Antimicrob Agents Chemother. 1996 Nov;40(11):2478-82.
16. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis. 1998 Jan;26(1):1-10.

17. Mouton JW, Dudley MN, Cars O, Derendorf H, Drusano GL. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. J Antimicrob Chemother. 2005 May;55(5):601-7.
18. Craig WA. Interrelationship between pharmacokinetics and pharmacodynamics in determining dosage regimens for broad-spectrum cephalosporins. Diagn Microbiol Infect Dis. 1995 May-Jun;22(1-2):89-96.
19. Drusano GL, Johnson DE, Rosen M, Standiford HC. Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of Pseudomonas sepsis. Antimicrob Agents Chemother. 1993 Mar;37(3):483-90.
20. Firsov AA, Vostrov SN, Shevchenko AA, Cornaglia G. Parameters of bacterial killing and regrowth kinetics and antimicrobial effect examined in terms of area under the concentration-time curve relationships: action of ciprofloxacin against Escherichia coli in an in vitro dynamic model. Antimicrob Agents Chemother. 1997 Jun;41(6):1281-7.
21. MacGowan A, Bowker K. Developments in PK/PD: optimising efficacy and prevention of resistance. A critical review of PK/PD in in vitro models. Int J Antimicrob Agents. 2002 Apr;19(4):291-8.
22. Eagle H, Fleischman R, Levy M. "Continuous" vs. "discontinuous" therapy with penicillin; the effect of the interval between injections on therapeutic efficacy. N Engl J Med. 1953 Mar 19;248(12):481-8.
23. Frimodt-Moller N, Bentzon MW, Thomsen VF. Experimental infection with Streptococcus pneumoniae in mice: correlation of in vitro activity and pharmacokinetic parameters with in vivo effect for 14 cephalosporins. J Infect Dis. 1986 Sep;154(3):511-7.
24. Gustafsson I, Lowdin E, Odenholt I, Cars O. Pharmacokinetic and pharmacodynamic parameters for antimicrobial effects of cefotaxime and amoxicillin in an in vitro kinetic model. Antimicrob Agents Chemother. 2001 Sep;45(9):2436-40.
25. Leggett JE, Fantin B, Ebert S, Totsuka K, Vogelman B, Calame W, et al. Comparative antibiotic dose-effect relations at several dosing intervals in murine pneumonitis and thigh-infection models. J Infect Dis. 1989 Feb;159(2):281-92.
26. Vogelman B, Gudmundsson S, Leggett J, Turnidge J, Ebert S, Craig WA. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. J Infect Dis. 1988 Oct;158(4):831-47.
27. Andes D, Craig WA. Pharmacodynamics of the new fluoroquinolone gatifloxacin in murine thigh and lung infection models. Antimicrob Agents Chemother. 2002 Jun;46(6):1665-70.
28. Craig WA, Kiem S, Andes DR. Free Drug 24-Hr AUC/MIC is the PK/PD Target that Correlates with In Vivo Efficacy of Macrolides, Azalides, Ketolides and Clindamycin. Abstract Interscience Conference on Antimicrobial Agents and Chemotherapy San Diego, 2002 (Abstract no A-1264).
29. Craig WA, Andes D. Pharmacokinetics and pharmacodynamics of antibiotics in otitis media. Pediatr Infect Dis J. 1996 Mar;15(3):255-9.
30. Ambrose PG, Grasela DM, Grasela TH, Passarell J, Mayer HB, Pierce PF. Pharmacodynamics of fluoroquinolones against Streptococcus pneumoniae in patients with community-acquired respiratory tract infections. Antimicrob Agents Chemother. 2001 Oct;45(10):2793-7.
31. Forrest A, Nix DE, Ballow CH, Goss TF, Birmingham MC, Schentag JJ. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. Antimicrob Agents Chemother. 1993 May;37(5):1073-81.

32. Moore RD, Lietman PS, Smith CR. Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. J Infect Dis. 1987 Jan;155(1):93-9.
33. Schentag JJ, Klugman KP, Yu VL, Adelman MH, Wilton GJ, Chiou CC, et al. Streptococcus pneumoniae bacteraemia: pharmacodynamic correlations with outcome and macrolide resistance--a controlled study. Int J Antimicrob Agents. 2007 Sep;30(3):264-9.
34. Moise-Broder PA, Forrest A, Birmingham MC, Schentag JJ. Pharmacodynamics of vancomycin and other antimicrobials in patients with Staphylococcus aureus lower respiratory tract infections. Clin Pharmacokinet. 2004;43(13):925-42.
35. Bradley JS, Dudley MN, Drusano GL. Predicting efficacy of antiinfectives with pharmacodynamics and Monte Carlo simulation. Pediatr Infect Dis J. 2003 Nov;22(11):982-92.
36. Mouton JW, Schmitt-Hoffmann A, Shapiro S, Nashed N, Punt NC. Use of Monte Carlo simulations to select therapeutic doses and provisional breakpoints of BAL9141. Antimicrob Agents Chemother. 2004 May;48(5):1713-8.
37. Owens RC, Jr., Bhavnani SM, Ambrose PG. Assessment of pharmacokinetic-pharmacodynamic target attainment of gemifloxacin against Streptococcus pneumoniae. Diagn Microbiol Infect Dis. 2005 Jan;51(1):45-9.
38. EMEA. Points to Consider on Pharmacokinetics and Pharmacodynamics in the Development of Antibacterial Medicinal Products. CPMP/EWP/2655/99. 2000.
39. Danhof M, de Lange EC, Della Pasqua OE, Ploeger BA, Voskuyl RA. Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling in translational drug research. Trends Pharmacol Sci. 2008 Apr;29(4):186-91.
40. Marshall S, Macintyre F, James I, Krams M, Jonsson NE. Role of mechanistically-based pharmacokinetic/pharmacodynamic models in drug development : a case study of a therapeutic protein. Clin Pharmacokinet. 2006;45(2):177-97.
41. Bulitta JB, Ly NS, Yang JC, Forrest A, Jusko WJ, Tsuji BT. Development and qualification of a pharmacodynamic model for the pronounced inoculum effect of ceftazidime against Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2009 Jan;53(1):46-56.
42. Udekwu KI, Parrish N, Ankomah P, Baquero F, Levin BR. Functional relationship between bacterial cell density and the efficacy of antibiotics. J Antimicrob Chemother. 2009 Apr;63(4):745-57.
43. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. Bacterial persistence as a phenotypic switch. Science. 2004 Sep 10;305(5690):1622-5.
44. Joers A, Kaldalu N, Tenson T. The frequency of persisters in Escherichia coli reflects the kinetics of awakening from dormancy. J Bacteriol. 2010 Jul;192(13):3379-84.
45. Keren I, Kaldalu N, Spoering A, Wang Y, Lewis K. Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett. 2004 Jan 15;230(1):13-8.
46. Czock D, Keller F. Mechanism-based pharmacokinetic-pharmacodynamic modeling of antimicrobial drug effects. J Pharmacokinet Pharmacodyn. 2007 Dec;34(6):727-51.
47. Regoes RR, Wiuff C, Zappala RM, Garner KN, Baquero F, Levin BR. Pharmacodynamic functions: a multiparameter approach to the design of antibiotic treatment regimens. Antimicrob Agents Chemother. 2004 Oct;48(10):3670-6.

48. Liu P, Rand KH, Obermann B, Derendorf H. Pharmacokinetic-pharmacodynamic modelling of antibacterial activity of cefpodoxime and cefixime in in vitro kinetic models. Int J Antimicrob Agents. 2005 Feb;25(2):120-9.
49. Treyaprasert W, Schmidt S, Rand KH, Suvanakoot U, Derendorf H. Pharmacokinetic/pharmacodynamic modeling of in vitro activity of azithromycin against four different bacterial strains. Int J Antimicrob Agents. 2007 Mar;29(3):263-70.
50. de la Pena A, Grabe A, Rand KH, Rehak E, Gross J, Thyroff-Friesinger U, et al. PK-PD modelling of the effect of cefaclor on four different bacterial strains. Int J Antimicrob Agents. 2004 Mar;23(3):218-25.
51. Zhi J, Nightingale CH, Quintiliani R. A pharmacodynamic model for the activity of antibiotics against microorganisms under nonsaturable conditions. J Pharm Sci. 1986 Nov;75(11):1063-7.
52. Mouton JW, Vinks AA, Punt NC. Pharmacokinetic-pharmacodynamic modeling of activity of ceftazidime during continuous and intermittent infusion. Antimicrob Agents Chemother. 1997 Apr;41(4):733-8.
53. Tam VH, Schilling AN, Nikolaou M. Modelling time-kill studies to discern the pharmacodynamics of meropenem. J Antimicrob Chemother. 2005 May;55(5):699-706.
54. Tam VH, Ledesma KR, Vo G, Kabbara S, Lim TP, Nikolaou M. Pharmacodynamic modeling of aminoglycosides against Pseudomonas aeruginosa and Acinetobacter baumannii: identifying dosing regimens to suppress resistance development. Antimicrob Agents Chemother. 2008 Nov;52(11):3987-93.
55. Gregoire N, Raherison S, Grignon C, Comets E, Marliat M, Ploy MC, et al. Semimechanistic pharmacokinetic-pharmacodynamic model with adaptation development for time-kill experiments of ciprofloxacin against Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2010 Jun;54(6):2379-84.
56. Meagher AK, Forrest A, Dalhoff A, Stass H, Schentag JJ. Novel pharmacokinetic-pharmacodynamic model for prediction of outcomes with an extended-release formulation of ciprofloxacin. Antimicrob Agents Chemother. 2004 Jun;48(6):2061-8.
57. Bulitta JB, Yang JC, Yohonn L, Ly NS, Brown SV, D'Hondt RE, et al. Attenuation of colistin bactericidal activity by high inoculum of Pseudomonas aeruginosa characterized by a new mechanism-based population pharmacodynamic model. Antimicrob Agents Chemother. 2010 May;54(5):2051-62.
58. Campion JJ, McNamara PJ, Evans ME. Pharmacodynamic modeling of ciprofloxacin resistance in Staphylococcus aureus. Antimicrob Agents Chemother. 2005 Jan;49(1):209-19.
59. Gumbo T, Louie A, Deziel MR, Parsons LM, Salfinger M, Drusano GL. Selection of a moxifloxacin dose that suppresses drug resistance in Mycobacterium tuberculosis, by use of an in vitro pharmacodynamic infection model and mathematical modeling. J Infect Dis. 2004 Nov 1;190(9):1642-51.
60. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet. 2010 Jun 5;375(9730):1969-87.
61. Muller-Pebody B, Johnson AP, Heath PT, Gilbert RE, Henderson KL, Sharland M. Empirical treatment of neonatal sepsis: are the current guidelines adequate? Arch Dis Child Fetal Neonatal Ed. 2011 Jan;96(1):F4-8.

62. Schentag JJ, Jusko WJ. Renal clearance and tissue accumulation of gentamicin. Clin Pharmacol Ther. 1977 Sep;22(3):364-70.
63. Laskin OL, Longstreth JA, Smith CR, Lietman PS. Netilmicin and gentamicin multidose kinetics in normal subjects. Clin Pharmacol Ther. 1983 Nov;34(5):644-50.
64. Heimann G. Renal toxicity of aminoglycosides in the neonatal period. Pediatr Pharmacol (New York). 1983;3(3-4):251-7.
65. Hardman JG, Limbird LE, Gilman AG. Goodman & Gilman's The Pharmacological Basis of Therapeutics - 10th ed. 2001.
66. Friis-Hansen B. Changes in body water compartments during growth. Acta Paediatr Suppl. 1957 Jan;46(suppl 110):1-68.
67. Besunder JB, Reed MD, Blumer JL. Principles of drug biodisposition in the neonate. A critical evaluation of the pharmacokinetic-pharmacodynamic interface (Part I). Clin Pharmacokinet. 1988 Apr;14(4):189-216.
68. Touw DJ, Westerman EM, Sprij AJ. Therapeutic drug monitoring of aminoglycosides in neonates. Clin Pharmacokinet. 2009;48(2):71-88.
69. Gordon RC, Regamey C, Kirby WM. Serum protein binding of the aminoglycoside antibiotics. Antimicrob Agents Chemother. 1972 Sep;2(3):214-6.
70. Rosario MC, Thomson AH, Jodrell DI, Sharp CA, Elliott HL. Population pharmacokinetics of gentamicin in patients with cancer. Br J Clin Pharmacol. 1998 Sep;46(3):229-36.
71. Alcorn J, McNamara PJ. Pharmacokinetics in the newborn. Adv Drug Deliv Rev. 2003 Apr 29;55(5):667-86.
72. van den Anker JN. Pharmacokinetics and renal function in preterm infants. Acta Paediatr. 1996 Dec;85(12):1393-9.
73. van der Heijden AJ, Grose WF, Ambagtsheer JJ, Provoost AP, Wolff ED, Sauer PJ. Glomerular filtration rate in the preterm infant: the relation to gestational and postnatal age. Eur J Pediatr. 1988 Oct;148(1):24-8.
74. van den Anker JN, Hop WC, de Groot R, van der Heijden BJ, Broerse HM, Lindemans J, et al. Effects of prenatal exposure to betamethasone and indomethacin on the glomerular filtration rate in the preterm infant. Pediatr Res. 1994 Nov;36(5):578-81.
75. Leake RD, Trygstad CW, Oh W. Inulin clearance in the newborn infant: relationship to gestational and postnatal age. Pediatr Res. 1976 Aug;10(8):759-62.
76. van den Anker JN, Schoemaker RC, Hop WC, van der Heijden BJ, Weber A, Sauer PJ, et al. Ceftazidime pharmacokinetics in preterm infants: effects of renal function and gestational age. Clin Pharmacol Ther. 1995 Dec;58(6):650-9.
77. Fawer CL, Torrado A, Guignard JP. Maturation of renal function in full-term and premature neonates. Helv Paediatr Acta. 1979 Feb;34(1):11-21.
78. Rubin MI, Bruck E, Rapoport M. Maturation of renal function in childhood; clearance studies. J Clin Invest. 1949 Sep;28(5 Pt. 2):1144-62.
79. Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. J Am Soc Nephrol. 2009 Nov;20(11):2305-13.
80. Price CP, Finney H. Developments in the assessment of glomerular filtration rate. Clin Chim Acta. 2000 Jul;297(1-2):55-66.
81. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.
82. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new

prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999 Mar 16;130(6):461-70.
83. Nolte S, Mueller B, Pringsheim W. Serum alpha 1-microglobulin and beta 2-microglobulin for the estimation of fetal glomerular renal function. Pediatr Nephrol. 1991 Sep;5(5):573-7.
84. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C--a new marker of glomerular filtration rate in children independent of age and height. Pediatrics. 1998 May;101(5):875-81.
85. Grubb A, Nyman U, Bjork J, Lindstrom V, Rippe B, Sterner G, et al. Simple cystatin C-based prediction equations for glomerular filtration rate compared with the modification of diet in renal disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. Clin Chem. 2005 Aug;51(8):1420-31.
86. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function--a review. Clin Chem Lab Med. 1999 Apr;37(4):389-95.
87. Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? Clin Chem. 2002 May;48(5):699-707.
88. Bokenkamp A, Dieterich C, Dressler F, Muhlhaus K, Gembruch U, Bald R, et al. Fetal serum concentrations of cystatin C and beta2-microglobulin as predictors of postnatal kidney function. Am J Obstet Gynecol. 2001 Aug;185(2):468-75.
89. Anderson BJ, Holford NH. Mechanistic basis of using body size and maturation to predict clearance in humans. Drug Metab Pharmacokinet. 2009;24(1):25-36.
90. West GB, Brown JH, Enquist BJ. The fourth dimension of life: fractal geometry and allometric scaling of organisms. Science. 1999 Jun 4;284(5420):1677-9.
91. Holford NH. A size standard for pharmacokinetics. Clin Pharmacokinet. 1996 May;30(5):329-32.
92. Blaser J, Stone BB, Groner MC, Zinner SH. Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. Antimicrob Agents Chemother. 1987 Jul;31(7):1054-60.
93. Turnidge J. Pharmacodynamics and dosing of aminoglycosides. Infect Dis Clin North Am. 2003 Sep;17(3):503-28.
94. Kashuba AD, Nafziger AN, Drusano GL, Bertino JS, Jr. Optimizing aminoglycoside therapy for nosocomial pneumonia caused by gram-negative bacteria. Antimicrob Agents Chemother. 1999 Mar;43(3):623-9.
95. Daikos GL, Lolans VT, Jackson GG. First-exposure adaptive resistance to aminoglycoside antibiotics in vivo with meaning for optimal clinical use. Antimicrob Agents Chemother. 1991 Jan;35(1):117-23.
96. Daikos GL, Jackson GG, Lolans VT, Livermore DM. Adaptive resistance to aminoglycoside antibiotics from first-exposure down-regulation. J Infect Dis. 1990 Aug;162(2):414-20.
97. Barclay ML, Begg EJ, Chambers ST. Adaptive resistance following single doses of gentamicin in a dynamic in vitro model. Antimicrob Agents Chemother. 1992 Sep;36(9):1951-7.
98. Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. Antimicrob Agents Chemother. 1999 May;43(5):1003-12.
99. Giuliano RA, Verpooten GA, Verbist L, Wedeen RP, De Broe ME. In vivo uptake kinetics of aminoglycosides in the kidney cortex of rats. J Pharmacol Exp Ther. 1986 Feb;236(2):470-5.

100. Giuliano RA, Verpooten GA, De Broe ME. The effect of dosing strategy on kidney cortical accumulation of aminoglycosides in rats. Am J Kidney Dis. 1986 Nov;8(5):297-303.
101. Dahlgren JG, Anderson ET, Hewitt WL. Gentamicin blood levels: a guide to nephrotoxicity. Antimicrob Agents Chemother. 1975 Jul;8(1):58-62.
102. Goodman EL, Van Gelder J, Holmes R, Hull AR, Sanford JP. Prospective comparative study of variable dosage and variable frequency regimens for administration of gentamicin. Antimicrob Agents Chemother. 1975 Oct;8(4):434-8.
103. Rougier F, Ducher M, Maurin M, Corvaisier S, Claude D, Jelliffe R, et al. Aminoglycoside dosages and nephrotoxicity: quantitative relationships. Clin Pharmacokinet. 2003;42(5):493-500.
104. Beaubien AR, Ormsby E, Bayne A, Carrier K, Crossfield G, Downes M, et al. Evidence that amikacin ototoxicity is related to total perilymph area under the concentration-time curve regardless of concentration. Antimicrob Agents Chemother. 1991 Jun;35(6):1070-4.
105. Contopoulos-Ioannidis DG, Giotis ND, Baliatsa DV, Ioannidis JP. Extended-interval aminoglycoside administration for children: a meta-analysis. Pediatrics. 2004 Jul;114(1):e111-8.
106. Nestaas E, Bangstad HJ, Sandvik L, Wathne KO. Aminoglycoside extended interval dosing in neonates is safe and effective: a meta-analysis. Arch Dis Child Fetal Neonatal Ed. 2005 Jul;90(4):F294-300.
107. Rao SC, Ahmed M, Hagan R. One dose per day compared to multiple doses per day of gentamicin for treatment of suspected or proven sepsis in neonates. Cochrane Database Syst Rev. 2006(1):CD005091.
108. Barza M, Ioannidis JP, Cappelleri JC, Lau J. Single or multiple daily doses of aminoglycosides: a meta-analysis. BMJ. 1996 Feb 10;312(7027):338-45.
109. Lanao JM, Calvo MV, Mesa JA, Martin-Suarez A, Carbajosa MT, Miguelez F, et al. Pharmacokinetic basis for the use of extended interval dosage regimens of gentamicin in neonates. J Antimicrob Chemother. 2004 Jul;54(1):193-8.
110. Thomson AH, Way S, Bryson SM, McGovern EM, Kelman AW, Whiting B. Population pharmacokinetics of gentamicin in neonates. Dev Pharmacol Ther. 1988;11(3):173-9.
111. Garcia B, Barcia E, Perez F, Molina IT. Population pharmacokinetics of gentamicin in premature newborns. J Antimicrob Chemother. 2006 Aug;58(2):372-9.
112. Weber W, Kewitz G, Rost KL, Looby M, Nitz M, Harnisch L. Population kinetics of gentamicin in neonates. Eur J Clin Pharmacol. 1993;44 Suppl 1:S23-5.
113. Rocha MJ, Almeida AM, Afonso E, Martins V, Santos J, Leitao F, et al. The kinetic profile of gentamicin in premature neonates. J Pharm Pharmacol. 2000 Sep;52(9):1091-7.
114. Jensen PD, Edgren BE, Brundage RC. Population pharmacokinetics of gentamicin in neonates using a nonlinear, mixed-effects model. Pharmacotherapy. 1992;12(3):178-82.
115. Botha JH, du Preez MJ, Adhikari M. Population pharmacokinetics of gentamicin in South African newborns. Eur J Clin Pharmacol. 2003 Dec;59(10):755-9.
116. Begg EJ, Vella-Brincat JW, Robertshawe B, McMurtrie MJ, Kirkpatrick CM, Darlow B. Eight years' experience of an extended-interval dosing protocol for gentamicin in neonates. J Antimicrob Chemother. 2009 May;63(5):1043-9.

117. Ette EI, Williams PJ. Pharmacometrics: the science of quantitative pharmacology. Hoboken, New Jersey: John Wiley & Sons, Inc. 2007.
118. Grasela TH, Sheiner LB. Pharmacostatistical modeling for observational data. J Pharmacokinet Biopharm Suppl 1991;19(3):25S-36S.
119. Tod M, Jullien V, Pons G. Facilitation of drug evaluation in children by population methods and modelling. Clin Pharmacokinet. 2008;47(4):231-43.
120. Larsson A, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. Scand J Clin Lab Invest. 2004;64(1):25-30.
121. Flodin M, Jonsson AS, Hansson LO, Danielsson LA, Larsson A. Evaluation of Gentian cystatin C reagent on Abbott Ci8200 and calculation of glomerular filtration rate expressed in mL/min/1.73 m(2) from the cystatin C values in mg/L. Scand J Clin Lab Invest. 2007;67(5):560-7.
122. Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J. Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. Clin Pharmacol Ther. 1979 Mar;25(3):358-71.
123. Karlsson MO, Beal SL, Sheiner LB. Three new residual error models for population PK/PD analyses. J Pharmacokinet Biopharm. 1995 Dec;23(6):651-72.
124. Anderson BJ, Holford NH. Mechanism-based concepts of size and maturity in pharmacokinetics. Annu Rev Pharmacol Toxicol. 2008;48:303-32.
125. Knibbe CA, Zuideveld KP, Aarts LP, Kuks PF, Danhof M. Allometric relationships between the pharmacokinetics of propofol in rats, children and adults. Br J Clin Pharmacol. 2005 Jun;59(6):705-11.
126. Lindbom L, Pihlgren P, Jonsson EN. PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Programs Biomed. 2005 Sep;79(3):241-57.
127. Beal SL. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn. 2001 Oct;28(5):481-504.
128. Wahlby U, Jonsson EN, Karlsson MO. Assessment of actual significance levels for covariate effects in NONMEM. J Pharmacokinet Pharmacodyn. 2001 Jun;28(3):231-52.
129. Beal SL, Sheiner LB, Boeckmann AJ. NONMEM Users Guides. Icon Development Solutions, Ellicott City, Maryland, USA. 1989-2006.
130. Karlsson MO, Holford N. A Tutorial on Visual Predictive Checks. PAGE 17. 2008. Abstr 1434 [http://www.page-meeting.org/?abstract=1434].
131. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm. 1981 Aug;9(4):503-12.
132. Ebert SC, Leggett J, Vogelman B, Craig WA. Evidence for a slow elimination phase for penicillin G. J Infect Dis. 1988 Jul;158(1):200-2.
133. Simon N, Sampol E, Albanese J, Martin C, Arvis P, Urien S, et al. Population pharmacokinetics of moxifloxacin in plasma and bronchial secretions in patients with severe bronchopneumonia. Clin Pharmacol Ther. 2003 Oct;74(4):353-63.
134. Viberg A, Lannergard A, Larsson A, Cars O, Karlsson MO, Sandstrom M. A population pharmacokinetic model for cefuroxime using cystatin C as a marker of renal function. Br J Clin Pharmacol. 2006 Sep;62(3):297-303.
135. Welling PG, Craig WA. Pharmacokinetics of intravenous erythromycin. J Pharm Sci. 1978 Aug;67(8):1057-9.
136. Yasuhara M, Iga T, Zenda H, Okumura K, Oguma T, Yano Y, et al. Population pharmacokinetics of vancomycin in Japanese pediatric patients. Ther Drug Monit. 1998 Dec;20(6):612-8.

137. Muller AE, DeJongh J, Bult Y, Goessens WH, Mouton JW, Danhof M, et al. Pharmacokinetics of penicillin G in infants with a gestational age of less than 32 weeks. Antimicrob Agents Chemother. 2007 Oct;51(10):3720-5.
138. Nielsen EI, Sandstrom M, Honore PH, Ewald U, Friberg LE. Developmental pharmacokinetics of gentamicin in preterm and term neonates: population modelling of a prospective study. Clin Pharmacokinet. 2009;48(4):253-63.
139. MacGowan A, Rogers C, Holt HA, Wootton M, Bowker K. Assessment of different antibacterial effect measures used in in vitro models of infection and subsequent use in pharmacodynamic correlations for moxifloxacin. J Antimicrob Chemother. 2000 Jul;46(1):73-8.
140. Jonsson EN, Karlsson MO. Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed 1999;58:51-64.
141. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. Comput Methods Programs Biomed. 2004 Aug;75(2):85-94.
142. Yano Y, Oguma T, Nagata H, Sasaki S. Application of logistic growth model to pharmacodynamic analysis of in vitro bactericidal kinetics. J Pharm Sci. 1998 Oct;87(10):1177-83.
143. Katsube T, Yano Y, Yamano Y, Munekage T, Kuroda N, Takano M. Pharmacokinetic-pharmacodynamic modeling and simulation for bactericidal effect in an in vitro dynamic model. J Pharm Sci. 2008 Sep;97(9):4108-17.
144. Schmidt S, Sabarinath SN, Barbour A, Abbanat D, Manitpisitkul P, Sha S, et al. Pharmacokinetic-pharmacodynamic modeling of the in vitro activities of oxazolidinone antimicrobial agents against methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2009 Dec;53(12):5039-45.
145. Lowdin E, Odenholt I, Cars O. In vitro studies of pharmacodynamic properties of vancomycin against Staphylococcus aureus and Staphylococcus epidermidis. Antimicrob Agents Chemother. 1998 Oct;42(10):2739-44.
146. DiCenzo R, Forrest A, Slish JC, Cole C, Guillet R. A gentamicin pharmacokinetic population model and once-daily dosing algorithm for neonates. Pharmacotherapy. 2003 May;23(5):585-91.
147. Craig WA, Andes D. Correlation of the Magnitude of the AUC[24]/MIC for 6 Fluoroquinolones against Streptococcus pneumoniae (SP) with Survival and Bactericidal Activity in an Animal Model. Abstract Interscience Conference on Antimicrobial Agents and Chemotherapy Toronto, 2000 (Abstract no 289).
148. Lacy MK, Lu W, Xu X, Tessier PR, Nicolau DP, Quintiliani R, et al. Pharmacodynamic comparisons of levofloxacin, ciprofloxacin, and ampicillin against Streptococcus pneumoniae in an in vitro model of infection. Antimicrob Agents Chemother. 1999 Mar;43(3):672-7.

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