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Pharmacokinetic/Pharmacodynamic (PK/PD) Indices of Antibiotics Predicted by a Semimechanistic PKPD Model: a Step toward Model-Based Dose Optimization▽

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A pharmacokinetic-pharmacodynamic (PKPD) model that characterizes the full time course of *in vitro* time-kill curve experiments of antibacterial drugs was here evaluated in its capacity to predict the previously determined PK/PD indices. Six drugs (benzylpenicillin, cefuroxime, erythromycin, gentamicin, moxifloxacin, and vancomycin), representing a broad selection of mechanisms of action and PK and PD characteristics, were investigated. For each drug, a dose fractionation study was simulated, using a wide range of total daily doses given as intermittent doses (dosing intervals of 4, 8, 12, or 24 h) or as a constant drug exposure. The time course of the drug concentration (PK model) as well as the bacterial response to drug exposure (*in vitro* PKPD model) was predicted. Nonlinear least-squares regression analyses determined the PK/PD index (the maximal unbound drug concentration [fC_{max}]/MIC, the area under the unbound drug concentration-time curve [*f*AUC]/MIC, or the percentage of a 24-h time period that the unbound drug concentration exceeds the MIC [$fT_{>MIC}$]) that was most predictive of the effect. The *in silico* predictions based on the *in vitro* PKPD model identified the previously determined PK/PD indices, with $fT_{>MIC}$ being the best predictor of the effect for β-lactams and *f*AUC/MIC being the best predictor for the four remaining evaluated drugs. The selection and magnitude of the PK/PD index were, however, shown to be sensitive to differences in PK in subpopulations, uncertainty in MICs, and investigated dosing intervals. In comparison with the use of the PK/PD indices, a model-based approach, where the full time course of effect can be predicted, has a lower sensitivity to study design and allows for PK differences in subpopulations to be considered directly. This study supports the use of PKPD models built from *in vitro* time-kill curves in the development of optimal dosing regimens for antibacterial drugs.

The pharmacokinetic (PK) and pharmacodynamic (PD) properties of a drug need to be well defined in order to make informed decisions regarding appropriate dosing recommendations. The regulatory authorities also clearly recommend that investigations of the PKPD relationship for antibacterial agents are included in drug development programs (25, 26). However, most of the antibacterial drugs on the market today were developed several decades ago and were not part of a modern drug development program. The currently used dosing regimens for these agents are generally based on point estimates of the effect (i.e., in terms of the MIC) and summary endpoints, such as the PK/PD indices. However, today, modern technology allows us to use more computer-intensive methods, and we have the possibility to explore more complex PKPD relationships. Model- and simulation-based methods are increasingly used within most therapeutic areas and provide for a quantitative description of the time course of drug effects, which offers great potential for achieving a more optimal drug therapy (8, 40, 60).

Historically, the MIC has been the major PD marker used in guiding the dosing of antibacterial drugs. Earlier, for all antibiotics, the dose was selected so that the plasma concentration of the drug exceeded the MIC for as long as possible. In the last decades, studies using *in vitro* and animal models have played an essential role in reaching a more detailed understanding regarding the relationship between the PK and PD properties of antibacterial agents (3, 13, 22, 27, 44). Commonly, a dose fractionation study design is applied, where a fixed total daily dose is administered as a single dose or fractionated into smaller doses administered using different dosing intervals. Based on such studies, the antibacterial drugs have been classified according to the correlation between the effect (most often defined as the bacterial count after 24 h of treatment) and the three PK/PD indices: (i) the ratio of the maximal unbound (free) drug concentration to the MIC (fC_{max}/MIC), (ii) the ratio of the area under the unbound drug concentration-time curve to the MIC (*f*AUC/MIC), or (iii) the percentage of a 24-h time period that the unbound drug concentration exceeds the MIC ($fT_{>MIC}$) (3, 13, 50). A vast number of studies have been conducted with different infection models with the aim of identifying the PK/PD index that best predicts the effect for various combinations of antibacterial agents and pathogens. Based on such studies, the activities of β-lactam antibiotics have been classified as being mostly dependent on the $fT_{>MIC}$ (12, 23, 29, 33, 42, 72), while the effects of the aminoglycosides and fluoroquinolones are correlated either to the fC_{max}/MIC or *f*AUC/MIC (4, 22, 42, 72). For the glycopeptides and macrolides, the PK/PD indices have been

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TABLE 1. MICs, $Cu_{ss,av}$ range used in simulations, and PK parameters used for simulating unbound drug concentration-time profiles in a typical patient

Drug	MIC (mg/liter)	$Cu_{ss,av}$ (mg/liter)	Unbound fraction[i]	CL (liter/h)	V_C (liter)	V_{P1} (liter)	V_{P2} (liter)	Q_{P1} (liter/h)	Q_{P2} (liter/h)
Benzylpenicillin[a]	0.012[g]	0.0000936–3.07	0.40	25.3	7.77	6.92	8.08	19.3	2.33
Cefuroxime[b]	0.016[g]	0.000125–4.10	0.67	7.16	11.4	5.11		3.65	
Erythromycin[c]	0.125[g]	0.000975–32	0.16	22.8	34.9	20.7		16.9	
Gentamicin[d]	2[h]	0.0156–512	1.0	4.38	7.73	9.51	15.0	3.49	0.113
Moxifloxacin[e]	0.125[g]	0.000975–32	0.61	16.1	63.2	64.7		21.6	
Vancomycin[f]	0.25[g]	0.00195–64	0.70	4.06	17.6	43.4		9.24	

[a] Represents a healthy volunteer with a body weight of 70 kg (24).
[b] Represents a patient with a cystatin C concentration of 1.12 mg/liter and a body weight of 74 kg (71).
[c] Represents a healthy volunteer with a body weight of 70 kg (73). Raw data in the publication by Welling and Craig (73) were remodeled using a two-compartment model with interindividual variability in CL and V_{P1} and a proportional residual-error model.
[d] Represents a healthy volunteer with a body weight of 72 kg (41).
[e] Represents a patient with a body weight of 76 kg (65).
[f] Represents a Japanese patient with a creatinine CL of 85 ml/min and a body weight of 55 kg (74).
[g] Against *Streptococcus pyogenes*.
[h] Against *Escherichia coli*.
[i] Values are from reference 34.

reported to differ among various agents of these drug classes. For erythromycin and vancomycin, the $fT_{>MIC}$ was initially reported to be a better predictor of the antibacterial effect than the fC_{max}/MIC and *f*AUC/MIC, based on their time-dependent killing pattern seen *in vitro*. However, more-recent studies, using only clinically relevant dosing regimens, have indicated that the effect is better correlated to the *f*AUC/MIC (13, 16, 72).

The PK/PD indices are frequently used as targets in the dose selection process. In Monte Carlo simulations, between-patient variability in PK parameters, as well as in PD (in terms of the MIC), is considered and the probability of the target attainment (PTA) is determined based on these stochastic simulations from the model (10, 52, 58). However, these PK/PD indices are summary endpoints, and in the process of creating such endpoints, detailed information about the time course of the individual PK and PD processes are lost. Models characterizing the full time course of PK as well as PD would provide a step forward toward a more accurate description of the antibacterial drug effect for different treatments and subpopulations.

With mechanistic models, prior knowledge and experience of the studied system are incorporated to increase the predictive ability of the model (21, 46). However, fully mechanistic models often become too complex, with too many parameters to characterize. A semimechanistic (also known as mechanism-based) approach is therefore applied, where mechanistic knowledge is utilized but the model is kept simple enough to allow for parameter estimation. The use of *in vitro* methods to study the PD of antibiotics has a long history (30), and lately these methods have regained interest when used in combination with a mechanism-based modeling approach (see, e.g., references 11, 18, 31, 53, 57, 61, 66, 69, and 75). Mechanism-based models developed from *in vitro* data provide for a quantitative description of the multiple processes that occur within a bacterial system, e.g., the change in the concentration-time profile of the drug, the bacterial growth and killing kinetics, and possible adaptations in bacterial susceptibility in response to the antibiotic exposure (47, 55, 57). Such PKPD models therefore provide a better understanding of these processes and offer great potential for achieving a more optimal drug therapy. However, before these *in vitro* PKPD models can be fully utilized, it should be confirmed that predictions based on the models are in agreement with the previously determined PK and PD characteristics of the antibacterial agents.

The aim of the present study was to evaluate the predictive capacity of previously developed semimechanistic *in vitro* PKPD models with respect to the identification of (i) the currently used PK/PD indices and (ii) the magnitudes of the selected PK/PD indices required to achieve a bacteriostatic and bactericidal effect. A secondary aim was to challenge the methodology used in the establishment of the PK/PD indices and thereby their consistency across different study conditions. This was performed by investigating the sensitivity of the PK/PD indices to factors such as different PK profiles, uncertainty in MIC, and choice of PD endpoint. The predictions in this study were based on previously presented *in vitro* PKPD models characterizing the growth and killing kinetics either of a *Streptococcus pyogenes* strain exposed to benzylpenicillin, cefuroxime, erythromycin, moxifloxacin, and vancomycin (55, 57) or of an *Escherichia coli* strain exposed to gentamicin (47).

MATERIALS AND METHODS

Antibiotics and bacteria. The antibacterial drugs investigated in this study included benzylpenicillin, cefuroxime, erythromycin, gentamicin, moxifloxacin, and vancomycin. The PKPD models used in the simulations are based on previously performed *in vitro* time-kill curve experiments described in detail elsewhere (47, 55, 57). In short, in one set of experiments, a strain of *S. pyogenes* group A (strain M12, NCTC P1800) was exposed benzylpenicillin, cefuroxime, erythromycin, moxifloxacin, or vancomycin. The drug exposure was either static (0 to 64× MIC) or dynamic where the initial drug concentration (2 and 16× MIC) decreased over time, with half-lives similar to those observed in patients. The PKPD model was developed based on the time-kill curve experiments with static drug concentrations (57) and was subsequently verified to be applicable also to dynamic concentration-time exposures (55). In a second set of experiments, a strain of *E. coli* (ATCC 25922) was exposed to static as well as dynamic concentration-time profiles of gentamicin (47). The MIC values of the six antibiotics are presented in Table 1. The MICs of benzylpenicillin, erythromycin, moxifloxacin, and vancomycin were determined by the Etest (Biodisk AB, Solna, Sweden) on Iso-Sensitest agar. For cefuroxime and gentamicin, the MICs were determined by the macrodilution technique according to the instructions of the Clinical and Laboratory Standards Institute (formerly the NCCLS). The MIC

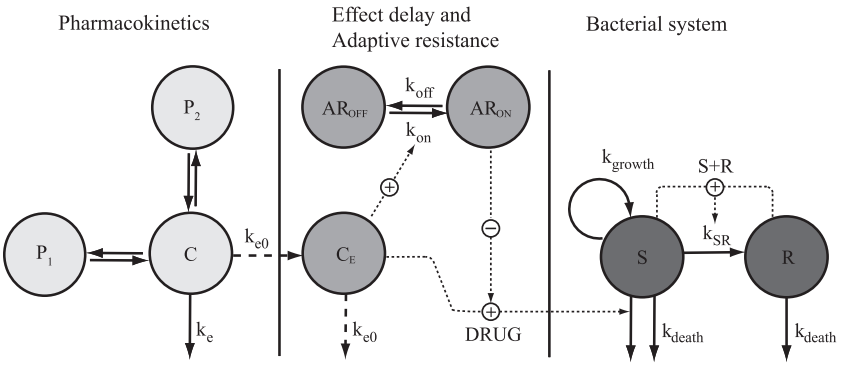

FIG. 1. Schematic illustration of the full semimechanistic PKPD model describing the time course of the drug concentration and the bacterial growth and killing after antibacterial treatment, as well as the development of adaptive resistance. C, central drug compartment; P_1 and P_2, peripheral drug compartments (P_2 is used only for benzylpenicillin and gentamicin); C_E, drug effect compartment; AR_{OFF} and AR_{ON}, compartments describing the development of adaptive resistance (only used for gentamicin); S, proliferating and drug-sensitive bacteria; R, resting and drug-insensitive bacteria; k_e, drug elimination rate constant; k_{e0}, rate constant for effect delay; 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; $k_{on,}$ rate constant for the development of adaptive resistance; $k_{off,}$ rate constant for return to susceptibility. The effective drug concentration (C_E) increases the death of bacteria in the drug-sensitive stage (S) according to a sigmoidal E_{max} function (DRUG). For gentamicin, C_E also drives the resistance development (AR_{ON}), and AR_{ON} reduces the E_{max} parameter of the E_{max} function.

values were in good agreement with, e.g., the EUCAST-reported MIC distributions (www.eucast.org). In this study, a lower MIC was used for cefuroxime than in the original publication (0.016 instead of 0.031 mg/liter). The lower MIC was regarded as more consistent with the results in the time-kill experiments as well as with previously reported values (43, 57).

Simulated dosing regimens. A dose fractionation study was simulated for each of the six drugs. A wide range of total daily doses ($n = 16$) were simulated to be administered using dosing intervals of 4, 8, 12, and 24 h. The simulated doses correspond to the daily doses needed to achieve an average unbound plasma drug concentration at steady state ($Cu_{ss,av}$) of 0.0078 to 256× MIC for the respective drug (Table 1). Included in the simulations were also constant drug exposures that yielded steady-state concentrations corresponding to those obtained by the 16 different intermittent dosing regimens. This wide range of doses was used to explore the full concentration-effect relationship for each dosing interval. Benzylpenicillin, cefuroxime, and gentamicin were simulated to be administered as intravenous bolus injections, and erythromycin, moxifloxacin, and vancomycin were administered as continuous intravenous infusions over 60 min. A common clinically used total daily dose in adults was also included for each dose interval and drug and were as follows: 6,000 mg, 2,250 mg, 1,200 mg, 315 mg, 400 mg, and 2,000 mg daily for benzylpenicillin, cefuroxime, erythromycin, gentamicin, moxifloxacin, and vancomycin, respectively.

Default simulation settings. (a) Pharmacokinetics. We searched the literature for compartmental PK models describing the plasma concentration-time profile of the six investigated drugs. The PK parameters, in terms of clearances (CL), intercompartmental clearances for the first and second peripheral compartments (Q_{P1} and Q_{P2}, respectively), volumes of distributions of the central drug compartment (C) and the peripheral compartments (V_C, V_{P1}, V_{P2}), and the unbound fractions used in the default simulation setting for the six drugs, are presented in Table 1. In the selection of PK models, multicompartment pharmacokinetic models were selected over one-compartment models since they were considered to give a more accurate representation of the plasma concentration-time profile. Other factors that influenced the model selection were the patient population, the number of patients, and the number and timing of PK samplings. For benzylpenicillin and gentamicin, three-compartment PK models were used to simulate the human concentration-time profile (24, 41). For cefuroxime, erythromycin, moxifloxacin, and vancomycin, two-compartment PK models were used (65, 71, 73, 74). For cefuroxime, moxifloxacin, and vancomycin, the PK studies were performed in patients with symptoms and signs of bacterial infection, patients with severe bronchopneumonia, and Japanese patients infected with methicillin-resistant *Staphylococcus aureus*, respectively. For benzylpenicillin, erythromycin, and gentamicin, PK models describing the full concentration-time profile were restricted to the characterization of data from studies performed with healthy volunteers.

(b) Pharmacodynamics. Previously described semimechanistic PKPD models (Fig. 1) were used to predict the bacterial killing kinetics following the different dosing regimens (47, 55, 57). The PK part of the model consisted of the central drug compartment (C) and either one or two peripheral compartments (P1, P2), depending on the drug (equations 1 to 3). In these equations, A is the amount of drug in each compartment.

$$dA_C/dt = -\mathrm{CL}/V_C \cdot A_C - Q_{P1}/V_C \cdot A_C + Q_{P1}/V_{P1} \cdot A_{P1} - Q_{P2}/V_C \cdot A_C + Q_{P2}/V_{P2} \cdot A_{P2} \quad (1)$$

$$dA_{P1}/dt = Q_{P1}/V_C \cdot A_C - Q_{P1}/V_{P1} \cdot A_{P1} \quad (2)$$

$$dA_{P2}/dt = Q_{P2}/V_C \cdot A_C - Q_{P2}/V_{P2} \cdot A_{P2} \quad (3)$$

An effect compartment (E) with a hypothetical drug concentration (C_E) was used to account for a time delay in the observed effect for erythromycin and moxifloxacin (equation 4).

$$dC_E/dt = k_{e0} \cdot C_C - k_{e0} \cdot C_E \quad (4)$$

This effect delay was characterized by a first-order rate constant (k_{e0}), and the effect compartment was introduced without affecting the mass balance of the concentrations in the kinetic compartments (64).

Two bacterial stages were included in the model: one proliferating and drug-sensitive stage (S) and one nongrowing and drug-insensitive stage (R) (equations 5 and 6).

$$dS/dt = k_{growth} \cdot S - (k_{death} + \mathrm{DRUG}) \cdot S - k_{SR} \cdot S \quad (5)$$

$$dR/dt = k_{SR} \cdot S - k_{death} \cdot R \quad (6)$$

Bacteria in S were assumed to have a net growth rate determined by a rate constant for multiplication of bacteria (k_{growth}) and a rate constant for the natural death of bacteria (k_{death}). Bacteria in R show no growth, but they are assumed to have the same natural death rate (k_{death}) as the bacteria in S. A start inoculum of 10^6 CFU/ml was used in all simulations. In this inoculum, the majority of the bacteria are in the growing drug-sensitive stage, but as the total bacterial content in the system increases, bacteria are transferred to the resting stage. This transfer was mediated by a rate constant (k_{SR}) which was implemented as a linear function of the total bacterial content in the system (equation 7), where B_{max} is the maximum achievable bacterial count in the system.

$$k_{SR} = \frac{(k_{growth} - k_{death})}{B_{max}} \cdot (S + R) \quad (7)$$

The drugs have been characterized to increase the death of the bacteria in the drug-sensitive stage according to a sigmoidal E_{max} function (equation 8), where E_{max} determines the maximum increased killing rate of the bacteria in the susceptible stage, EC_{50} is the effect compartment concentration (C_E) of the antibiotic that produces 50% of the E_{max}, and γ is the sigmoidicity factor characterizing the steepness of the concentration-effect relationship.

TABLE 2. Drug-specific PD parameters used for simulations of bacterial counts

Drug	E_{max} (h^{-1})	EC_{50} (mg/liter)	γ	k_{e0} (h^{-1})[a]
Benzylpenicillin	2.70	0.00531	1.06	—
Cefuroxime	2.72	0.00787	1.35	—
Erythromycin	2.46	0.0336	0.652	1.02
Gentamicin	51.0[c]	9.93	1[b]	—
Moxifloxacin	3.40	0.0750	1.42	0.627
Vancomycin	1.52	0.304	4.99	—

[a] —, no time delay; k_{e0} was fixed at 10,000.
[b] Simple E_{max} model; γ was fixed at 1.
[c] Represents the $E_{max(0)}$, i.e., the E_{max} when no adaptive resistance has been developed.

$$\text{DRUG} = \frac{E_{max} \cdot C_E^{\gamma}}{C_E^{\gamma} + EC_{50}^{\gamma}} \quad (8)$$

Following an initial exposure to aminoglycoside antibiotics, such as gentamicin, bacteria generally show a period of adaptive or phenotypic resistance. This is a reversible form of resistance, shown both *in vitro* and *in vivo*, where short-term gentamicin exposures result in a transient period of reduced killing activity in an originally susceptible bacterial population (6, 19, 20). To describe the reduced drug sensitivity due to this adaptive resistance, the PKPD model used for the gentamicin predictions was modified to also include a binding model with the on and off adaptive-resistance states (AR_{ON} and AR_{OFF}, respectively) (equations 9 and 10).

$$dAR_{ON}/dt = k_{on} \cdot AR_{OFF} \cdot C_E - k_{off} \cdot AR_{ON} \quad (9)$$

$$dAR_{OFF}/dt = -k_{on} \cdot AR_{OFF} \cdot C_E + k_{off} \cdot AR_{ON} \quad (10)$$

Initially, the amount in AR_{OFF} was set to 1 and the amount in AR_{ON} was 0; i.e., no degree of adaptive resistance was present. Two rate constants describe the development (k_{on}) and reversal (k_{off}) of adaptive resistance, respectively. The gentamicin concentration (C_E) drives the resistance development (k_{on}), and the amount (fraction) that has transferred from AR_{OFF} to AR_{ON} reduces the E_{max} of gentamicin from an initial value [$E_{max(0)}$] by a nonlinear function (equation 11).

$$E_{max} = E_{max(0)} \cdot \left(1 - \frac{AR_{ON}}{AR_{ON} + AR_{50}}\right) \quad (11)$$

AR_{50} describes the amount in AR_{ON} needed to reduce the $E_{max(0)}$ by 50%. Note that equations 9 to 11 are for gentamicin only.

(c) Parameter values in default simulation setting. In the default setting, the drug concentration in the central PK compartment caused the death of the drug-sensitive bacteria. The bacterial count after 24 h of drug exposure was used as the PD endpoint. For benzylpenicillin, cefuroxime, erythromycin, moxifloxacin, and vancomycin, the bacterium-specific parameters used in the simulations were for k_{growth}, k_{death}, and B_{max}: 1.46 h^{-1}, 0.187 h^{-1}, and 5.00×10^8 CFU/ml, respectively, reflecting parameter estimates for *S. pyogenes* (55). For gentamicin, the bacterium-specific parameters were for k_{growth}, k_{death}, and B_{max}: 2.00 h^{-1}, 0.179 h^{-1}, and 8.26×10^8 CFU/ml, respectively, reflecting parameter estimates for *E. coli* (47). Parameters used to describe the adaptive resistance of gentamicin were for k_{on}, k_{off}, and AR_{50}: 0.0426 liter mg^{-1} h^{-1}, 0.0139 h^{-1}, and 0.113, respectively (A. F. Mohamed, E. I. Nielsen, O. Cars, and L. E. Friberg, unpublished data). To be able to describe the rapid and extensive bacterial killing effect for gentamicin, k_{SR} was set to 0 if the predicted total bacterial count was less than an estimated breakpoint, 2.09×10^6 CFU/ml (Mohamed et al., unpublished). Drug-specific PD parameters used in the simulations are presented in Table 2. Simulations were performed without variability; i.e., the simulations are predictions based on typical structural model parameters.

(d) Evaluation of PK/PD indices. 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 using the predicted unbound drug concentrations in combination with the respective MIC (Table 1) (50). The fC_{max} reflects the highest unbound drug concentration which for intermittent dosing is reached at the time of the bolus injection or at the end of the infusion. For the constant drug exposure, the fC_{max} equals the $Cu_{ss,av}$. The fAUC was defined as the area under the unbound drug concentration-time curve over the first 24 h of treatment (i.e., any residual drug exposure after 24 h was not considered) and was determined by integration of the unbound drug concentration-time curve. The $fT_{>MIC}$ was determined as the cumulative percentage of the first 24 h of treatment that the unbound drug concentration exceeded the MIC and was computed directly within the NONMEM code. If the effect (PD endpoint) was evaluated prior to 24 h (e.g., the $\log_{10}$ CFU/ml at 12 h, see below), the fAUC/MIC and $fT_{>MIC}$ up to the time of the effect evaluation was used (e.g., fAUC$_{12}$/MIC or $fT_{12>MIC}$).

The relationship between the effect and the three PK/PD indices was evaluated according to a sigmoidal E_{max} type function as follows:

$$E = E_0 - \frac{PD_{max} \cdot X^{Hill}}{X^{Hill} + EX_{50}^{Hill}} \quad (12)$$

where E is the PD endpoint (e.g., bacterial count, as $\log_{10}$ CFU/ml, after 24 h of treatment), E_0 is the baseline effect representing the value of the PD endpoint without drug treatment (i.e., when the PK/PD index is 0), X is the three PK/PD indices as defined above, PD_{max} is the maximum effect, EX_{50} is the magnitude of X that is needed to achieve 50% of the E_{max}, and Hill is the sigmoidicity factor, reflecting the steepness of the relationship. For each drug and PK/PD index, all data from the five different dosing regimens were fit simultaneously; i.e., a total of 6×3 fitting procedures were performed in the default setting. Curve fitting was performed in R using the nonlinear least-squares (nls) algorithm. 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 endpoint of antibacterial effect. The magnitudes of the selected PK/PD index that were required to achieve a bacteriostatic or a bactericidal effect were calculated in R based on estimated parameters from the nls fit. A bacteriostatic effect (B_{stat}) was defined as an unchanged bacterial count at 24 h compared with the initial inoculum (i.e., a bacterial count of 10^6 CFU/ml at 24 h), while a bactericidal effect (B_{cid}) was defined as a 3-$\log_{10}$ decrease in bacterial count (99.9% killing) at 24 h compared with the initial inoculum.

Altered simulation settings. To investigate the sensitivity to altered PK or PD characteristics on the selection and magnitudes of the PK/PD index required for bacteriostatic and bactericidal effects, the same type of effect predictions and PK/PD index evaluations as in the default setting were made in four different altered simulation settings. The four settings (described in detail below) evaluated the sensitivity to the parameters of (i) the PK model, (ii) the choice of MIC value, (iii) the choice of antibacterial effect measure, and (iv) the choice of a central or peripheral PK compartment to drive the concentration-effect relationship.

(i) Modification of the pharmacokinetics. The selection of the PK/PD index and the magnitude of the PK/PD index needed for effect are often assumed to be the same across different species and/or patient subpopulations (54, 59, 68). In order to test that assumption, predictions from the model were performed where *(a)* CL was reduced to 1/3 of its original value and *(b)* the PK model was altered to characterize the concentration-time profile for benzylpenicillin and gentamicin in preterm neonates with a gestational age of 30 weeks on day 3 of life (54, 56). In the simulation settings with reduced CL values, all doses were scaled by the difference in CLs to reach the same $Cu_{ss,av}$ and AUC as in the default setting.

(ii) Uncertainty in MIC. The determination of the MIC most often involves using a 2-fold-dilution concentration series. This is a method with a high inherent uncertainty, and when the measured MIC is used as the PD characteristic in the PK/PD indices, this poor precision propagates into the PK/PD indices. The sensitivity to the measured MIC value was investigated by changing the MIC to *(a)* a one-step dilution up or down, i.e., 0.5×MIC or 2× MIC, respectively, *(b)* the EC_{50}s estimated earlier (47, 55), and *(c)* an MIC value predicted based on the *in vitro* PKPD model. The model-predicted MIC value was evaluated to minimize the influence of the lack of precision in the MIC determination in the calculation of the magnitude of the PK/PD indices. The macrobroth dilution experimental setup was mimicked, and the calculated MIC was determined as the exact static concentration (adjusted for *in vitro* drug degradation) that after an incubation period of 20 h resulted in a bacterial count of 10^7 CFU/ml using a start inoculum of 5×10^5 CFU/ml. The calculated MIC values were 0.0047, 0.0078, 0.027, 1.3, 0.055, and 0.37 mg/liter for benzylpenicillin, cefuroxime, erythromycin, gentamicin, moxifloxacin, and vancomycin, respectively.

(iii) Alternative PD endpoints. In the *in vitro* time-kill curve experiments, the bacterial count over time represents the magnitude of the effect. In previous characterizations of the PD of antibacterial agents, several PD endpoints have been suggested (27, 45), and the choice of endpoint might influence the conclusions regarding the PK/PD index. In the default simulation setting, the bacterial count after 24 h of drug exposure was used as the summary PD endpoint. The following alternative PD endpoints were also investigated in this study: *(a)* the bacterial count after 12, 48, and 240 h of exposure; *(b)* the time needed

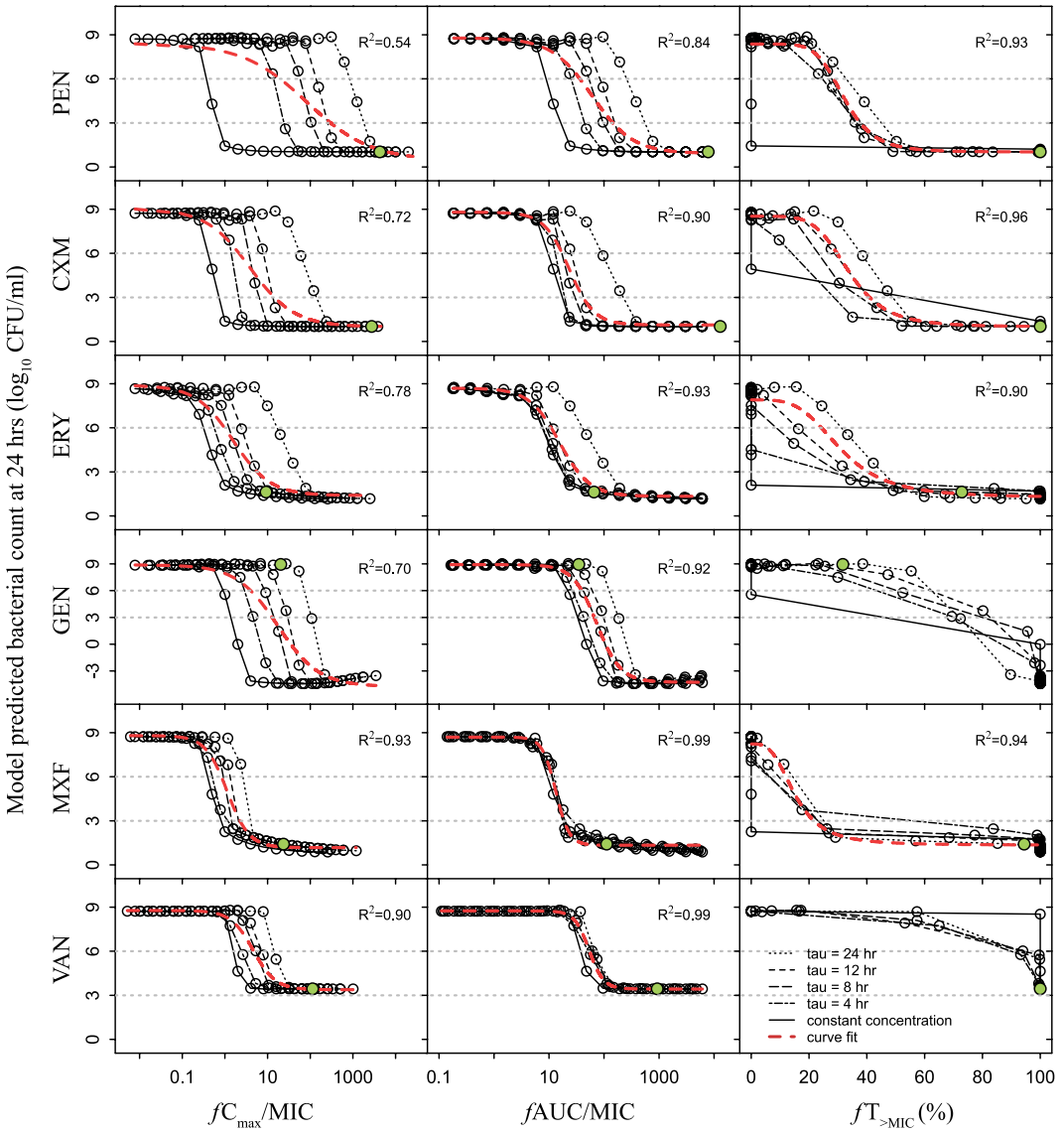

FIG. 2. Relationship between the model-predicted bacterial count after 24 h of therapy ($\log_{10}$ CFU/ml at 24 h) and the pharmacodynamic indices (unbound C_{max}/MIC ratio, unbound AUC/MIC ratio, and the percentage of time that the unbound drug plasma concentration exceed the MIC) after treatment 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. Included are the nonlinear least-squares regression line (dashed red line), the coefficient of determination (R^2), and lines representing a bacteriostatic and bactericidal effect (horizontal dotted gray lines). The predictions for clinically commonly used dosing regimens (1,000 mg × 6, 750 mg × 3, 400 mg × 3, 315 mg × 1, 400 mg × 1, and 1,000 mg × 2 for benzylpenicillin, cefuroxime, erythromycin, gentamicin, moxifloxacin, and vancomycin, respectively) are indicated in green. tau, the dosing interval used in the simulations.

for the initial inoculum to be reduced 100-fold ($T_{99\%}$), 1,000-fold ($T_{99.9\%}$), or 10,000-fold ($T_{99.99\%}$); and *(c)* the area under the $\log_{10}$ of the bacterial count curve from 0 to 24 h ($AUBC_{24}$) or 0 to 48 h ($AUBC_{48}$).

(iv) Modification of the concentration driving the antibacterial effect. In the default setting, the drug concentration in the central PK compartment (*C*) was assumed to drive the antibacterial effect. This resembles a systemic bacteremia, with the pathogen being located in the plasma compartment. The infection site could also be located peripherally. In such cases, the concentration in one of the peripheral compartments of the PK model could be more closely related to the effect. Therefore, the relationship between the antibacterial effect and the PK/PD indices was also determined in simulations where the concentration-time profile in one of the peripheral compartments (P1 or P2) drove the antibacterial effect.

Software. All simulations were performed within the population analysis software NONMEM VI (7). R 2.11.1 (www.r-project.org) was used to visualize the data, to perform nonlinear-curve fitting, and to calculate the coefficients of determination and log likelihood, as well as the values of the PK/PD indices required to achieve bacteriostatic and bactericidal effects. The model files used in the simulations can be provided by the authors upon request.

RESULTS

Results for model-predicted 24-h bacterial counts ($\log_{10}$ CFU/ml) versus the three PK/PD indices for each dosing regimen and drug are presented for three different scenarios in Fig. 2 to 4. The *x* axis for the fC_{max}/MIC and *f*AUC/MIC is log transformed for clarity, but no transformation of these indices was used in the data analysis. The bacterial counts were log

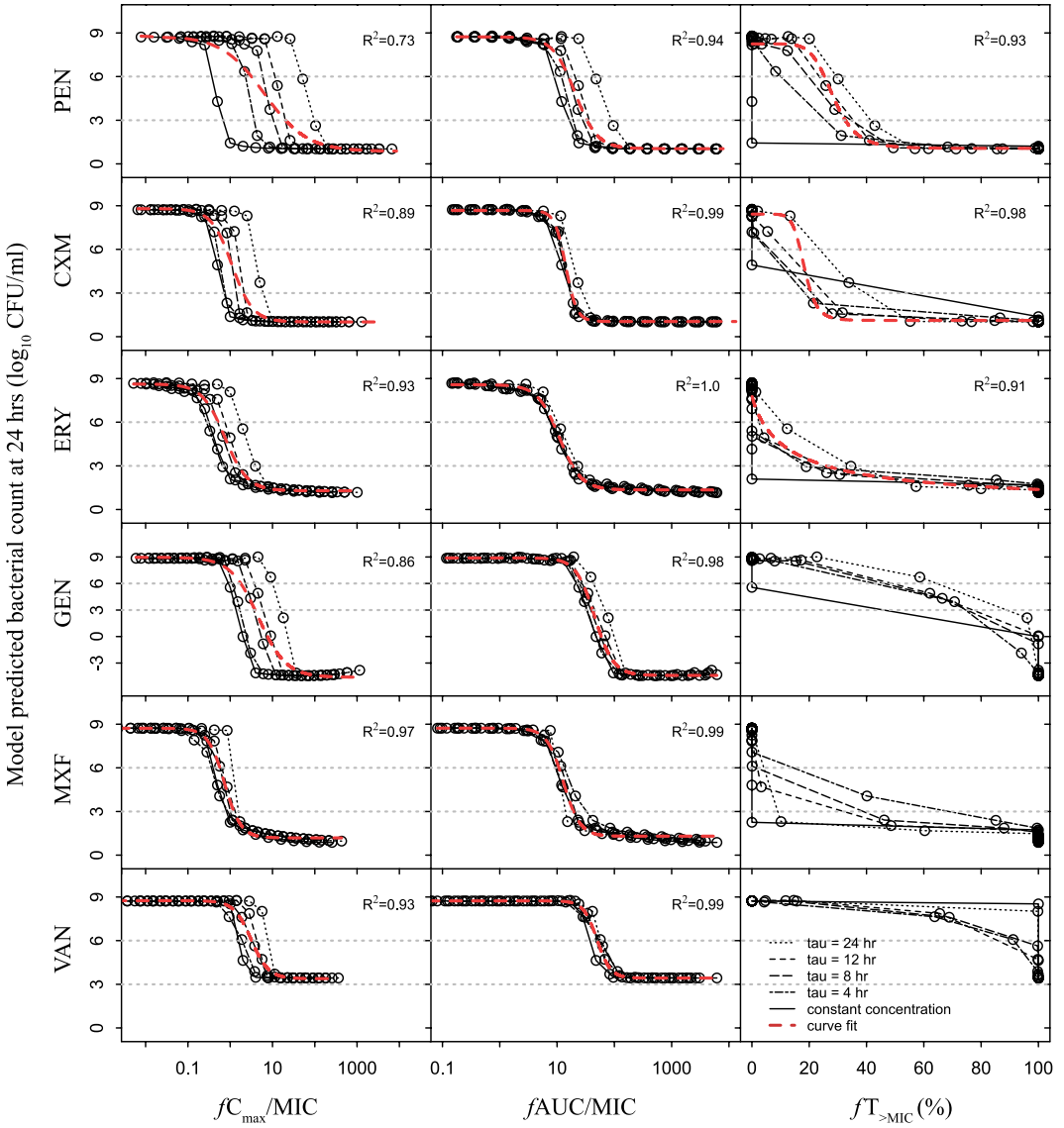

FIG. 3. Relationship between the model-predicted bacterial count after 24 h of therapy and the pharmacodynamic indices (fC_{max}/MIC, fAUC/MIC, and $fT_{>MIC}$) when a reduced CL (one-third of the original CL) was used in the simulation. Drugs: benzylpenicillin (PEN), cefuroxime (CXM), erythromycin (ERY), gentamicin (GEN), moxifloxacin (MXF), or vancomycin (VAN). Each simulated dosing regimen (i.e., dose and dosing intervals) are represented by one data point in each panel. Included are a nonlinear least-squares regression line (dashed red line), the coefficient of determination (R^2), and lines representing a bacteriostatic and bactericidal effect (horizontal dotted gray lines).

transformed prior to analysis. In the evaluation of the PK/PD index that was most predictive of the endpoint variable for effect, the R^2 and the log-likelihood approach yielded consistent results, and therefore only the R^2 values are reported for the investigated settings (Table 3).

Default simulation setting. Based on the simulations using the default setting, the $fT_{>MIC}$ was selected as the PK/PD index most predictive of the antibacterial effect for the β-lactam antibiotics benzylpenicillin and cefuroxime (Fig. 2). For the β-lactam antibiotics, an increased effect was observed for frequent drug administration or when drug exposure was constant. The magnitudes of the $fT_{>MIC}$ required to achieve bacteriostatic and bactericidal effects were estimated to be 29 and 38%, respectively, for benzylpenicillin and 30 and 41% for cefuroxime (Table 3). For erythromycin, gentamicin, moxifloxacin, and vancomycin, the fAUC/MIC was selected as the PK/PD index showing the highest correlation with the antibacterial effect (Fig. 2) and the effects of these drugs were hence less dependent on the dosing frequency. The magnitudes of fAUC/MIC ratios needed to achieve bacteriostatic and bactericidal effects were 11 and 34 for erythromycin, 39 and 67 for gentamicin, and 11 and 19 for moxifloxacin, respectively (Table 3). For vancomycin, a bactericidal effect was not reached during the first 24 h of drug exposure and a fAUC/MIC of 54 was predicted to be needed for a bacteriostatic effect.

Calculated magnitudes of the PK/PD indices using the standard dosing regimens were 100% of the $fT_{>MIC}$ for the β-lac-

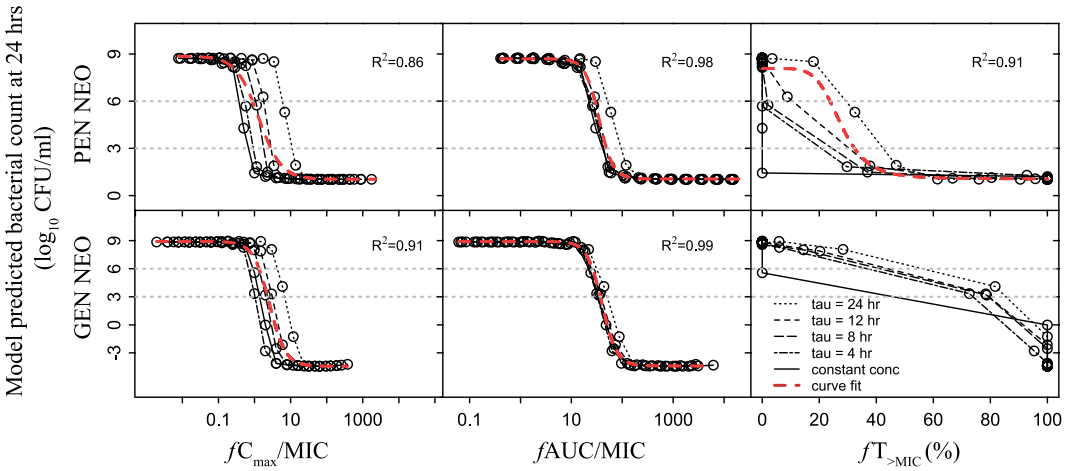

FIG. 4. Relationship between the model-predicted bacterial count after 24 h of therapy and the pharmacodynamic indices (fC_{max}/MIC, fAUC/MIC, and $fT_{>MIC}$) for neonates (NEO) (gestational age, 30 weeks; postnatal age, 3 days). PEN, benzylpenicillin; GEN, gentamicin. Each simulated dosing regimen (i.e., dose and dosing intervals) is represented by one data point in each panel. Included are a nonlinear least-squares regression line (dashed red line), the coefficient of determination (R^2), and lines representing bacteriostatic and bactericidal effects (horizontal dotted gray lines).

tams and fAUC/MIC ratios of 66, 35, 112, and 928 for erythromycin, gentamicin, moxifloxacin, and vancomycin, respectively. The standard dosing regimens were predicted to achieve a close-to-maximum effect for all drugs except for gentamicin (Fig. 2). For a gentamicin dosing regimen of 315 mg (4.5 mg/kg of body weight) given once daily, the fC_{max}/MIC was calculated to be 21 for the "true" peak concentration and 8.0 for the 1-h postinjection concentration. This dosing was predicted to result in an initial rapid killing effect, with bacterial counts as low as 50 CFU/ml 4 h after the initiation of therapy. However, bacterial regrowth was predicted to occur with bacterial counts close to B_{max} at 24 h.

The conclusions regarding the selection of the PK/PD index with the highest predictive value were not affected by reducing the multicompartment PK models to one-compartment models using the parameters describing the beta elimination phase ($t_{1/2\beta}$) in the predictions (data not shown). Further, the inclusion of an effect delay did not affect the selection of PK/PD indices (data not shown). The conclusions also remained unchanged when only doses corresponding to $Cu_{ss,av}$ values of 0.125 to 16× MIC were included in the evaluation (i.e., the number of total daily doses was reduced from 16 to 8).

Altered simulation settings. Coefficients of determination as well as magnitudes of the determined PK/PD indices required to achieve bacteriostatic and bactericidal effects for the altered simulation settings are presented in Table 3.

(i) Modification of the pharmacokinetics. *(a)* When CL was reduced to one-third of its original value, both the fC_{max}/MIC and fAUC/MIC showed higher correlations with the antibacterial endpoint for all investigated drugs (Fig. 3; Table 3). For benzylpenicillin and cefuroxime, the fAUC/MIC and $fT_{>MIC}$ showed similar correlations to the effect and a shorter $fT_{>MIC}$ was needed to achieve bacteriostatic and bactericidal effects in this simulation setting. Similarly, for erythromycin and gentamicin, a reduced fAUC/MIC was needed to reach the same effect when CL was reduced (Table 3). *(b)* In accordance with the results from *(a)*, both fC_{max}/MIC and fAUC/MIC became better predictors of the effect when the PK profile was simulated for premature neonates (Fig. 4). For benzylpenicillin, the fAUC/MIC showed a considerably higher correlation to the effect than the $fT_{>MIC}$ (R^2 = 0.98 compared to R^2 = 0.91). For gentamicin, the fAUC/MIC remained the index with best correlation to the effect, although the estimated magnitude required for bactericidal effect decreased from 67 to 37 (Table 3).

(ii) Uncertainty in MICs. The correlation between the $fT_{>MIC}$ and the effect was highly dependent on the MIC, and a change in the MIC had an influence on both the selection and the magnitude of the PK/PD index (Table 3). In general, a higher correlation between the $fT_{>MIC}$ and the effect was seen when we used the MIC values determined based on the actual time-kill data, i.e., an MIC set to the EC_{50} or calculated based on a model fit to the time-kill curve data. As expected, the change in MIC did not influence the correlations for the fC_{max}/MIC or fAUC/MIC when the PK was unchanged, and the magnitude of these PK/PD indices needed for the effect was changed in proportion to the MIC.

(iii) Alternative PD endpoints. The choice of PD endpoint was not found to have a large impact on the selection or on the magnitude of the PK/PD index (Table 3). The exception was gentamicin, for which the $fT_{>MIC}$ became a better predictor of the gentamicin effect than the fAUC/MIC when the PD endpoint was the time needed for the initial inoculum to be reduced by 100-fold ($T_{99\%}$), 1,000-fold ($T_{99.9\%}$), or 10,000-fold ($T_{99.99\%}$).

(iv) Modification of the concentration driving the antibacterial effect. In general, the correlation between the fC_{max}/MIC as well as the fAUC/MIC and the effect increased when the antimicrobial killing effect was driven by the antibiotic concentration in one of the peripheral compartments (Table 3). However, the conclusions regarding the best PK/PD index were the

TABLE 3. Coefficients of determination and magnitudes of the most correlated PK/PD index, as determined in the default setting, required for a bacteriostatic or bactericidal effect[a]

Simulation setting	Benzylpenicillin					Cefuroxime					Erythromycin				
	R^2			$fT_{>MIC}$		R^2			$fT_{>MIC}$		R^2			*f*AUC/MIC	
	fC_{max}/MIC	*f*AUC/MIC	$fT_{>MIC}$	B_{stat} (%)	B_{cid} (%)	fC_{max}/MIC	*f*AUC/MIC	$fT_{>MIC}$	B_{stat} (%)	B_{cid} (%)	fC_{max}/MIC	*f*AUC/MIC	$fT_{>MIC}$	B_{stat}	B_{cid}
Default	0.54	0.84	0.93*	29	38	0.72	0.90	0.96*	30	41	0.78	0.93*	0.90	11	34
i *(a)* Reduced CL	0.73	0.94*	0.93	25	33	0.89	0.99*	0.98	17	21	0.93	1.0*	0.91	8.5	19
(b) PK neonate	0.86	0.98*	0.91	24	32										
ii *(a)* 0.5× MIC	0.54	0.84	0.98*	40	52	0.72	0.90	0.98*	45	56	0.78	0.93	0.96*	22	67
2× MIC	0.54	0.84	0.88*	20	26	0.72	0.90*	0.87	15	31	0.78	0.93*	0.80	5.5	17
(b) Calculated MIC	0.54	0.84	0.99*	45	58	0.72	0.90	0.98*	46	57	0.78	0.93	0.94*	51	155
(c) EC_{50}	0.54	0.84	0.99*	42	55	0.72	0.90	0.98*	46	57	0.78	0.93	0.97*	41	125
iii *(a)* $\log_{10}$ CFU/ml (12 h)	0.66	0.92	0.94*	14	25	0.85	0.98*	0.98*	13	27	0.93	1.0*	0.92	5.1	32
$\log_{10}$ CFU/ml (48 h)	0.53	0.82	0.93*	28	33	0.71	0.89	0.96*	28	35	0.76	0.92*	0.88	8.8	19
$\log_{10}$ CFU/ml (240 h)	0.52	0.80	0.90*	27	29	0.67	0.86	0.92*	26	30	0.73	0.90*	0.80	6.9	9.3
(b) $AUBC_{24}$	0.68	0.94*	0.94*			0.86	0.99*	0.98			0.92	1.0*	0.92		
$AUBC_{48}$	0.62	0.90	0.94*			0.80	0.96	0.98*			0.86	0.98*	0.91		
(c) $T_{99\%}$	0.63	0.91	0.94*			0.81	0.97	0.99*			0.88	0.99*	0.96		
$T_{99.9\%}$	0.61	0.90	0.96*			0.80	0.97	0.98*			0.85	0.98*	0.95		
$T_{99.99\%}$	0.58	0.87	0.97*			0.76	0.94	0.99*			0.83	0.95*	NA		
iv C_{P1} driving PD	0.60	0.84	0.93*	31	38	0.82	0.93	0.97*	37	41	0.86	0.95*	0.89	10	32
C_{P2} driving PD	0.87	0.96*	0.92	32	38										

[a] Roman numerals and italic letters refer to sections in the text (see "Altered simulation settings" in Materials and Methods and in Results). *, the PK/PD index with the best correlation (highest R^2) to the PD endpoint; NA, not estimable.

same as for the default setting, except with benzylpenicillin, for which the *f*AUC/MIC was the best predictor when the effect was driven by the concentration in the second peripheral compartment. This is in accordance with the results in (i) above, since the peripheral compartments have lower fluctuations in the concentration-time profile.

DISCUSSION

In this simulation study, we have shown that PKPD models based on *in vitro* time-kill curve experiments can identify the previously determined PK/PD indices for six different antibacterial agents, with predicted magnitudes for the effect being similar to those observed *in vivo*. The choice and magnitude of the PK/PD index were, however, found to be sensitive to study conditions.

For the two β-lactam antibiotics investigated (benzylpenicillin and cefuroxime), the $fT_{>MIC}$ was the PK/PD index identified to be best correlated to the effect. This is in accordance with previous findings for numerous *in vitro* animal as well as clinical studies for a broad range of members of this drug class (15, 33, 42, 72). The magnitude of $fT_{>MIC}$s required for bactericidal effect was in this study estimated to be about 40%, which is also in agreement to previously reported values of 35 to 50% (12, 13, 15, 33). For the fluoroquinolone moxifloxacin, the *f*AUC/MIC was the best predictor of the effect, with an *f*AUC/MIC of 19 required for bactericidal effect. This value is close to previously reported *f*AUC/MIC breakpoints of 25 to 34 for the fluoroquinolones in the treatment of Gram-positive bacteria, as determined *in vitro* in animal models and in clinical studies (2, 14, 39). In animal models using Gram-negative bacteria and in seriously ill patients infected primarily with Gram-negative pathogens, higher breakpoints (AUC/MIC > 125 to 160) have been reported (28, 36).

For aminoglycosides, the *f*AUC/MIC has generally been referred to as the most important PK/PD index for the effect (70, 72), but the fC_{max}/MIC has also been suggested to be important (9, 35, 49). Previously reported breakpoints for fC_{max}/MIC and *f*AUC/MIC ratios of 8 to 10 and 80 to 100 (9, 37, 49, 70, 72), respectively, are in good agreement with the fC_{max}/MIC and *f*AUC/MIC ratios of 13 and 67, respectively, required for bactericidal effect in the present study. It should be noted that the *f*AUC/MIC and fC_{max}/MIC are highly correlated. Even though these targets are equal when it comes to dose adjustments due to differences in MICs (e.g., a 2-fold higher MIC requires a doubling of the dose for drugs with linear PK), this is not the case when it comes to adjustments due to differences in PK. For a 50% reduction in CL, a 50% dose reduction or a doubling of the dose interval would be implied when the *f*AUC/MIC is the target. However, if the fC_{max}/MIC is used as the target, no dose adjustment needs to be made for effect reasons since the C_{max} after bolus injections is unaffected by changes in CL unless drug accumulation occurs.

For erythromycin, both the *f*AUC/MIC and $fT_{>MIC}$ have been described to be good predictors of the effect, with reported breakpoints of 16 and 45 to 50%, respectively (62, 72). These findings correlate well with the results in this study where the *f*AUC/MIC and $fT_{>MIC}$ had similar R^2 values (0.93 and 0.90, respectively) and where the *f*AUC/MIC and $fT_{>MIC}$ needed for bactericidal effect were 11 and 41%, respectively. Previous studies with vancomycin have shown that an *f*AUC/MIC of 87.5 to 280 (AUC/MIC > 125 to 400) is associated with improved patient outcome (35, 48). In the current study, bactericidal effect was not achieved during the first 24 h of vancomycin treatment (i.e., a bacterial count of >1,000 CFU/ml at 24 h), but *f*AUC/MIC ratios of >54 were needed for a bacteriostatic effect.

The PK/PD indices are summary endpoints combining information regarding the PK and PD characteristics of a drug.

TABLE 3—*Continued*

Gentamicin					Moxifloxacin					Vancomycin				
R^2			*f*AUC/MIC		R^2			*f*AUC/MIC		R^2			*f*AUC/MIC	
fC_{max}/MIC	*f*AUC/MIC	$fT_{>MIC}$	B_{stat}	B_{cid}	fC_{max}/MIC	*f*AUC/MIC	$fT_{>MIC}$	B_{stat}	B_{cid}	fC_{max}/MIC	*f*AUC/MIC	$fT_{>MIC}$	B_{stat}	B_{cid}
0.70	0.92*	NA	39	67	0.93	0.99*	0.94	11	19	0.90	0.99*	NA	54	NA
0.86	0.98*	NA	28	42	0.97	0.99*	NA	11	19	0.93	0.99*	NA	51	NA
0.91	0.99*	NA	26	37										
0.71	0.92*	NA	79	133	0.93	0.99*	0.98	23	37	0.90	0.99*	NA	109	NA
0.71	0.92	0.94*	20	33	0.93	0.99*	0.85	5.7	9.4	0.90	0.99*	NA	27	NA
0.71	0.92*	NA	64	109	0.93	0.99*	0.98	26	42	0.90	0.99*	NA	37	NA
0.71	0.92*	0.88	8	14	0.93	0.99*	0.97	19	31	0.90	0.99*	NA	45	NA
0.80	0.94*	NA	8	21	0.96	0.99*	0.94	6	13	0.91	0.99*	NA	29	NA
0.73	0.94*	NA	51	70	0.92	0.99*	0.92	9.3	14	0.90	0.99*	NA	42	66
0.75	0.96*	NA	57	68	0.84*	NA	NA	NA	NA	0.86	0.98*	NA	39	41
0.82	0.97*	NA			0.97	0.99*	0.94			0.93	0.99*	NA		
0.78	0.95*	NA			0.95	0.99*	0.94			0.92	0.99*	NA		
0.79	0.95*	0.95*			0.96	0.98*	0.96			0.87	0.98*	NA		
0.75	0.94	0.96*			0.96	0.97*	0.93			NA	NA	NA		
0.71	0.92	0.97*			0.92	0.93*	NA			NA	NA	NA		
0.94	0.98*	NA	26	39	0.96	0.97*	NA	12	34	0.96	0.98*	NA	50	NA
0.97	0.99*	NA	23	33										

When used in Monte Carlo simulations to support dosing regimens, these indices and their magnitudes needed for the effect are, however, assumed to be independent of differences in PK and to be consistent between patient subpopulations. In the present study, the selection and magnitudes of the PK/PD indices were found to be dependent on the half-life of the drug. The β-lactam antibiotics had the shortest half-lives and were also the drugs for which the $fT_{>MIC}$ was the major predictor of the effect. In fact, when the PK profile of benzylpenicillin was used for all drugs (i.e., with all drugs assumed to have as short a half-life as benzylpenicillin), the $fT_{>MIC}$ was judged to be a better predictor of the effect than the other two PK/PD indices for all drugs, regardless of their PD parameters (data not shown). A high rate of elimination and intermittent dosing creates high fluctuations in the concentration-time profile, with high peak concentrations followed by prolonged periods of low drug levels, permitting regrowth of surviving bacteria. When the rate of elimination in the simulations was decreased to mimic what occurs in patient groups with lower clearances, the *f*AUC/MIC became a predictor of the effect that was at least as important as the $fT_{>MIC}$. The dependency of the PK/PD index on the half-life has previously been recognized in the determination of the PK/PD index. The $fT_{>MIC}$ was the PK/PD index most predictive of the effect for amikacin in neutropenic mice with normal renal function ($t_{1/2}$ = 20 min); however, when the half-life was increased to 2 h, the AUC/MIC became the most important PK/PD index (17). Another example is the macrolides, whose antimicrobial activities were initially regarded as being best related to the $fT_{>MIC}$, but when the data with the largest fluctuations, i.e., data from once or twice daily dosing in mice, were excluded, the *f*AUC/MIC was the selected PK/PD index (16).

The selection of the PK/PD index was shown to be dependent on study design, as the choice was sensitive to which dosing intervals were included in the evaluation. For example, if the data from 12- and 24-hourly dosing intervals were excluded, the *f*AUC/MIC became the best predictor also for the β-lactams, and hence the *f*AUC/MIC was a universal predictor of the effect for all six studied drugs, as has been suggested previously (63). In addition, constant infusions are not always included in these types of studies, and when the data from constant drug exposure in the current analysis was excluded, the correlation between each of the three PK/PD indices and the effect increased, and for erythromycin and moxifloxacin, the $fT_{>MIC}$ became as good a predictor of the effect as the *f*AUC/MIC (data not shown).

The selection and magnitude of the PK/PD index were in the present study shown to be sensitive to uncertainty in the MICs. The MIC is incorporated in the PK/PD indices as the descriptor of the PD. However, the use of the MIC has several disadvantages. It is measured as a threshold value, and the PD 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 with the MIC is that it is a summary measurement of all events that have taken place in the bacterial culture during the incubation period, including drug degradation and phenotypic and/or genetic resistance development. Further, the MIC is measured using 2-fold-concentration series, which have a high inherent poor precision with an upward bias (i.e., the "true" MIC is less than the reported value). These shortcomings with the MIC are propagated into the PK/PD indices, and the simulations performed in this study also highlight the sensitivity of the selection and magnitude of the PK/PD index to the uncertainty in the measurements of the MIC. Full characterization of the time course of antibacterial effect would overcome many of these drawbacks with the MIC as a PD marker and is therefore warranted in the development of dosing regimens.

Often, there is the assumption that there exists a "true" PK/PD index that is consistent within a drug class and among

species, pathogens, dosing regimens, and patient subpopulations, as long as variability in MIC, protein binding, and tissue distribution is accounted for (1, 3, 5). One shortcoming of these simulations is that the target PK/PD index is often assumed to be the same across patient populations with different PK characteristics. The simulations presented here clearly demonstrate that this is not the case. A shown in Fig. 4 for benzylpenicillin, a target *f*AUC/MIC value of 100 may be a better target (less dependent on the dosing regimen) in neonates than a target $fT_{>\mathrm{MIC}}$ of 40%, which is commonly used in the adult patient population. A more advantageous approach to develop dosing regimens would be to perform the Monte Carlo simulations on models with a full quantitative description of the time courses of all simultaneously occurring processes (e.g., PK, PD, resistance, etc.). Categorizations necessary for practical dose recommendations to different subpopulations and bacterial susceptibilities (MICs) could then be performed at the very end of the dose selection process and thereby minimize the loss of information and the use of potentially erroneous extrapolations.

The PKPD model utilized in this simulation study was originally built on data characterizing the interaction between *S. pyogenes* or *E. coli* and antimicrobial agents in an *in vitro* environment. The bacterial killing *in vivo* is also affected by host defense mechanisms, the pathology of the infection, and the virulence of the pathogen. Despite the difference between conditions, the magnitude of exposure associated with effect was predicted to be similar to those previously determined in animals and humans. The PKPD models used here were developed based on single bacterial strains. When data from several strains are available, the same methodology could be used to evaluate the consistency of these results and the PK/PD indices between bacterial strains.

The simulation approach presented in this study is most likely not restricted to the here-used PK and PKPD models. We used a previously developed PKPD model that has been shown to perform well for the simultaneous characterization of the antibacterial effects of several antibacterial agents belonging to different classes (47, 55, 57). A good agreement between *in vitro* model predictions and the commonly used *in vivo* targeted $fT_{>\mathrm{MIC}}$ have also been shown for the β-lactam antibiotics, using other model structures (38, 51). In this study, it seems not to have been of importance for the *in vitro-in vivo* comparison that the applied PKPD model does not predict the development of resistance (except for the adaptive resistance for gentamicin). By incorporating heterogeneous bacterial inocula, *in vitro* PKPD models have also been shown to be predictive of drug resistance (32, 67).

In conclusion, the simulations based on a model characterizing *in vitro* time-kill curve experiments successfully selected the previously determined PK/PD indices for six investigated antibacterial drugs belonging to different classes. We have also illustrated that PK/PD indices are not always consistent between patient subpopulations with different PK characteristics. This study supports mechanism-based PKPD modeling based on *in vitro* studies as a flexible and powerful tool to describe the effect of antibacterial agents, and such PKPD models are therefore suggested to be used in experimental and clinical study design and in the development of dosing guidelines. To be applicable in clinical practice, the guidelines will, however, be needed to be adapted to clinical measurements of bacterial susceptibility (such as the MIC), but the target level and optimal dosing regimen should be based on quantitative descriptions of the full time course of PK as well as PD and tailored to the population to be treated.

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