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Model-Informed Drug Development for Antimicrobials: Translational PK and PK/PD Modeling to Predict an Efficacious Human Dose for Apramycin

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Apramycin represents a subclass of aminoglycoside antibiotics that has been shown to evade almost all mechanisms of clinically relevant aminoglycoside resistance. Model-informed drug development may facilitate its transition from preclinical to clinical phase. This study explored the potential of pharmacokinetic/pharmacodynamic (PK/PD) modeling to maximize the use of *in vitro* time-kill and *in vivo* preclinical data for prediction of a human efficacious dose (HED) for apramycin. PK model parameters of apramycin from four different species (mouse, rat, guinea pig, and dog) were allometrically scaled to humans. A semimechanistic PK/PD model was developed from the rich *in vitro* data on four *Escherichia coli* strains and subsequently the sparse *in vivo* efficacy data on the same strains were integrated. An efficacious human dose was predicted from the PK/PD model and compared with the classical PK/PD index methodology and the aminoglycoside dose similarity. One-compartment models described the PK data and human values for clearance and volume of distribution were predicted to 7.07 L/hour and 26.8 L, respectively. The required *f*AUC/MIC (area under the unbound drug concentration-time curve over MIC ratio) targets for stasis and 1-log kill in the thigh model were 34.5 and 76.2, respectively. The developed PK/PD model predicted the efficacy data well with strain-specific differences in susceptibility, maximum bacterial load, and resistance development. All three dose prediction approaches supported an apramycin daily dose of 30 mg/kg for a typical adult patient. The results indicate that the mechanistic PK/PD modeling approach can be suitable for HED prediction and serves to efficiently integrate all available efficacy data with potential to improve predictive capacity.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The human efficacious dose (HED) is predicted based on preclinical data to support clinical drug development. The pharmacokinetic/pharmacodynamic (PK/PD) relationships of antibiotics are typically defined using the PK/PD index methodology.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ In this study, PK and efficacy data from *in vitro* and *in vivo* studies in preclinical drug development were combined for translational PK and PK/PD modeling to support HED prediction. The predicted dose was compared with the dose suggested from the classical PK/PD index methodology.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The results indicate that the PK/PD modeling approach can be suitable for HED prediction. Furthermore, the modeling allowed for integration of all available data mechanistically with predictive capacity not possible to achieve using the classical methodology.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This work has shown how PK/PD modeling can maximize the information gain from preclinical antibiotic studies on efficacy. The potential for model-informed drug development in the translation of antibiotics is demonstrated.

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Aminoglycosides are potent broad-spectrum antibiotics that bind to bacterial ribosomes to inhibit protein biosynthesis.[1,2] Apramycin is a monosubstituted deoxystreptamine and this uniquely distinctive chemical structure from other aminoglycosides minimizes the cross-resistance to apramycin.[3–6] In addition, apramycin has also been demonstrated to have a low potential for toxicity.[7,8] These properties make apramycin an attractive candidate for development into a new clinical therapy.

The human efficacious dose (HED) is predicted based on preclinical data to support clinical development. The drug exposure related to HED, and the drug exposure related the no-observed-adverse-effect level, define the anticipated therapeutic window. Hence, human dose estimation is dependent on reliable prediction of human pharmacokinetics (PK) and pharmacodynamics (PD).[9] To this end, predictions of human PK from PK parameters in preclinical species are made (e.g., based on allometric scaling).[10–12] This is the choice for drugs eliminated by the kidney because there is no reliable *in vitro* methods for predicting renal clearance.[13,14]

In the past decades, the PK/PD relationships of antibiotics have been described by evaluating the correlation of PK/PD indices (i.e., *f*AUC/MIC, fC_{max}/MIC, and *f*T>MIC), which are summary measures of drug exposure in relation to the minimum inhibitory concentration (MIC) of the drug, to an outcome variable, such as 24-hour response in an animal infection model.[15–18] From the index with the best correlation, a PK/PD target is derived. With a population PK model for humans and Monte–Carlo simulations, the probability of target attainment (PTA) in the virtual population, given the derived PK/PD targets, are evaluated for different dosing regimens.[15] These PK/PD indices are, however, simplifications of the PK/PD relationships neglecting the time-course of PK and PD. In addition, given the reliance on MIC, these PK/PD indices also suffer from the drawbacks of MICs (i.e., these are crude threshold values neglecting measurement error and the dynamic nature of bacterial growth and susceptibility).[19,20]

In vitro time course of bacterial growth and killing are used for development of semimechanistic PK/PD models.[15,21] In conjunction with the predicted human PK profiles, such PK/PD models have been used to predict alternative human dosages of available drugs.[9,22,23] In this study, PK and efficacy data from *in vitro* and *in vivo* studies in preclinical development were combined for translational PK and PK/PD modeling. The predicted dose from the modeling approach was compared with the doses predicted using the classical PK/PD index methodology based on PTA for stasis and scaling clinical aminoglycoside doses with typical differences in their MICs.

METHODS

Data collection

Pharmacokinetic studies. Animals (see **Supplementary Table S1** for details) were housed under standard conditions (21–23°C, reversed 12-hour light/dark cycle, and relative humidity 45–65%) with unlimited access to food and water. The experimental procedures were performed in accordance with the guidelines of the European Community and local laws and policies (Directive 2010/63/EU), and all of the procedures were approved by the Food and Veterinary Service, Riga, Latvia, and by the National Committee of Animal Ethics, Ministry of Environment and Food of Denmark. Concentrations of apramycin were measured in plasma samples from mice, rats, guinea pigs, and dogs following either s.c. or i.v. administration. Briefly, NMRI mice, Sprague-Dawley rats, and Hartley guinea pigs were given s.c. doses of apramycin, whereas beagle dogs were given i.v. doses of apramycin either as a bolus dose or 25-minute infusion. Plasma samples were collected at predetermined time points (**Supplementary Table S1**).

Plasma sample analyses were performed using a quantitative ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. Briefly, a sample volume of 10 µL was transferred into a plastic tube and mixed with 20 µL trichloroacetic acid (10% aqueous solution) to precipitate proteins. The mixture was diluted with 470 µL of mobile phase A (0.01% of heptafluorobutyric acid and 0.01% of propionic acid aqueous solution) and centrifuged at 20000x ***g*** for 10 minutes. Supernatant was subjected to UPLC-MS/MS analysis on an UPLC system Acquity H-class (Waters) connected with triple quadrupole mass spectrometer Xevo TQ-S (Waters). UPLC conditions: column-Acquity BEH C18 (2.1 × 50 mm, 1.7 µm); mobile phase A: 0.01% HFBA solution in 0.01% propionic acid aqueous solution, B: 0.01% HFBA solution in 0.01% propionic acid solution in acetonitrile; gradient initial 17%B, 0.5 minutes 17%B, 1.6 minutes 80%B, 3 minutes 80%B, 3.5 minutes 17%B, 5 minutes 17%B; flow 0.4 mL/minute; column temperature 40°C; and injection volume 1 µL. MS conditions: ionization electrospray ionization positive mode; capillary voltage 1.0 kV; electrospray ionization source temperature 120°C; desolvation gas (N2) flow 800 L/hour; and desolvation temperature 600°C. Multiple reaction monitoring parameters were as follows: m/z 540.2 → m/z 378.0 at cone voltage 80 V and collision energy 15 eV. The UPLC-MS/MS method quantified apramycin plasma concentrations in the range 1.28–1,315 µg/mL (mouse), 0.80–615 µg/mL (rat), 2.53–615 µg/mL (guinea pig), and 0.84 to 68 µg/mL (dog).

Time-kill experiments. Time-kill experiments were performed on four *Escherichia coli* strains (ATCC 25922, a reference strain for antibiotic susceptibility testing; ATCC 700336, an SXT-resistant urinary tract infection isolate; EN335, a multidrug-resistant ESBL-positive clinical isolate; and EN591, a multidrug-resistant *rmtB* isolate) with MICs ranging from 4 to 8 mg/L. Briefly, overnight cultures of each strain were diluted 100-fold into 2 mL pre-warmed Mueller Hinton II broth, grown 1.5 hours to logarithmic phase (OD600 0.1–0.3), then aliquots of ~ 10^6 bacteria were inoculated into polypropylene tubes containing 2 mL pre-warmed MHII medium pH 7.3. Apramycin was added to the tubes to achieve the intended concentration range (**Supplementary Table S2**). The tubes were incubated at 37°C and samples were taken for viable counts at 0, 1, 2, 4, 6, 8, 10, 24, and 28 hours. Appropriate dilutions of each sample were made in 0.9% NaCl and spread on MHII agar plates using glass beads (5 beads/plate, 6 mm diameter, Hecht 1401/6). After sampling, agar plates were incubated at 37°C for 18–24 hours before the number of colony-forming units (CFUs) was counted manually. The limit of detection was 10 CFU/mL.

***In vivo* efficacy studies in mice.** The protocols of the studies were approved by the local animal ethics committees (SSI, Denmark). Briefly, female NMRI mice (5–6 weeks old, 26–30 g, $N = 6$) were rendered neutropenic via i.p. injection of 2 doses of cyclophosphamide on day 4 and day 1 before infection. The animals were infected on day 0 via intramuscular injection of an inoculum containing 10^6 bacteria/mL in the left thigh. The same strains of *E. coli* were used as in the *in vitro* experiments. The animals were treated with apramycin ranging from 0.39 to 2,400 mg/kg/day starting at 1 hour after inoculation. The daily doses were fractionated into smaller doses for s.c. administration. Animals were either euthanized at the start of treatment or at the study end point after treatment initiation. Thighs were aseptically removed from the animals, homogenized, diluted, and plated

for incubation. Bacterial counting was performed after 18–22 hours of incubation at 35°C in ambient air.

PK/PD model development

PK modeling and prediction of human PK parameters. The PK parameters were estimated for each of the four species separately. Because only s.c. data were available for mice, rats, and guinea pigs, bioavailability (F) was set to 1 for estimation of the other PK parameters. Clearance (CL) and volume of distribution (V) of each animal were parameterized during the estimation, as shown in Eqs. 1 and 2:

$$CL_i = CL \cdot \left(\frac{WT}{70}\right)^{0.75} \quad (1)$$

$$V_i = V \cdot \left(\frac{WT}{70}\right)^{1} \quad (2)$$

where CL_i and V_i are the individual animal parameters and WT (kg) is the animal body weight. CL and V are the typical parameters of a human adult with a body weight of 70 kg.

One-compartment and two-compartment models with first-order absorption and linear elimination or saturable (nonlinear) elimination were evaluated. The observed dose-dependency in absorption in the mouse studies was explored by scaling the absorption rate constant (k_a) to the 30 mg/kg dose, as shown in Eq. 3, where k_{a30} is the k_a at 30 mg/kg and the power parameter *pow* was estimated.

$$k_a = k_{a30} \cdot \left(\frac{Dose}{30}\right)^{pow} \quad (3)$$

The PK parameters estimated from the animal species were used for allometric scaling to predict human CL and V of apramycin. Because human clearances are generally well-predicted using simple allometry for renally cleared drugs,[24] simple allometry was used as shown in Eq. 4, where CL_u is the clearance based on unbound drug, a is the allometric coefficient, b is the scaling exponent, and WT is the body weight.[10] CL and V parameters were corrected with the unbound fractions (f_us) in each animal species. The f_u values for mouse, rat, guinea pig, dog, and human had been determined to 0.58, 0.67, 0.59, 0.58, and 0.62, respectively (in-house data).

$$CL_u = a \cdot (WT)^b \quad (4)$$

PK/PD modeling. A PK/PD model was developed by first utilizing the *in vitro* time-kill data. Initially, strain-dependent models were developed and then all time-kill data were combined for simultaneous analysis. The model was adapted from an earlier model applied to gentamicin, another aminoglycoside antibiotic, and details of the equations used in the model can be found in an earlier publication.[25] To describe the regrowth of bacteria as observed in the time-kill data, adaptive resistance was included in the model.[26–29] The natural bacteria kill rate constant (k_{death}) was fixed to 0.179 hour^{-1} as in previous publications.[30]

The model developed from the *in vitro* time-kill data was then used to analyze the *in vivo* efficacy data by first fitting the model to the *in vivo* growth control data to estimate the *in vivo* growth rate constant and delay. The k_{growth} for *in vitro* and *in vivo* growth was parameterized, as shown in Eqs. 5 and 6:

$$k_{growth} = k_{g_vitro} \quad (5)$$

$$k_{growth} = k_{g_vivo} \cdot \frac{T^{\beta}}{T_{delay}^{\beta} + T^{\beta}} \quad (6)$$

where k_{g_vitro}, k_{g_vivo}, T, T_{delay}, and β represent the growth rate constant *in vitro*, the growth rate constant *in vivo*, the time since bacteria injection, the median duration of delay in growth, and the sigmoidicity factor, respectively. In the model, β was fixed to 5 to describe a gradual increase in growth of bacteria *in vivo*. To stabilize the model, system growth capacity *in vivo* (B_{max}) for each strain was fixed to the mean value observed from the growth control data at the study end point. Thereafter, *in vivo* efficacy data from the different dosing regimens were added to the model for analysis. Plasma concentrations of apramycin as predicted by the PK model for mice were converted to unbound concentrations to drive *in vivo* bacterial killing in the PK/PD model.

PK/PD indices

Data from 24-hour dose fractionation studies in the murine thigh infection model were available for the same four *E. coli* strains as those studied *in vitro*. The three PK/PD indices, *f*AUC/MIC, *f*T>MIC, and *f*C_{max}/MIC, were computed based on the PK parameters estimated for mice and correlated to the response at 24 hours after the start of treatment. The PK/PD targets of stasis and 1-log reduction were determined by composite regression analysis.

Human efficacious dose prediction

Given the human dosing recommendations for aminoglycosides in the literature,[31] only once-daily regimens were considered. For the PK/PD model-based approach, suitable doses were predicted for *E. coli* infections using the PK/PD model developed by finding the dose that predicts stasis. Unbound plasma concentrations in a typical patient with a creatinine clearance (CrCL) of 80 mL/minute (typical value of critically ill patients), as predicted from the allometrically scaled PK parameters, were driving the PK/PD model. For the PK/PD index approach, unbound concentration-time profiles of 1,000 virtual patients with a CrCL of 80 mL/minute were simulated from the population PK model of gentamicin[32] to perform the PTA analysis. For aminoglycoside similarity, clinically validated doses of aminoglycosides were scaled by the expected differences in their MICs, based on the ratios between the MIC_{90} of apramycin for Enterobacteriaceae (8 mg/L) and the clinical breakpoint/MIC values of gentamicin, plazomicin,[6] and amikacin according to EUCAST (www.eucast.org).

Data analysis and software

Model development was performed using the nonlinear mixed-effects modeling software NONMEM version 7.4 (ICON Development Solutions, San Antonio, TX), with Perl-speaks-NONMEM,[33] and the Laplacian conditional estimation method with interaction. R (version 3.5; R Foundation for Statistical Computing, Vienna, Austria) with the Xpose package was used for data management and graphical evaluation.[34] The data were log-transformed for modeling (i.e., transform-both-sides-approach). Data below the lower limit of detection were handled using the likelihood-based M3 method.[35] Interindividual variability was not estimated.

The likelihood ratio test was used to evaluate statistical significance for inclusion of additional parameters, where the objective function value (OFV) is assumed to be χ^2 distributed. For nested models with one parameter difference, a change in OFV (dOFV) of ≥ 3.84 was considered as a statistical difference at the 5% significance level (i.e., $P < 0.05$, for 1 degree of freedom). For residual unexplained variability, proportional error models, as approximated by log-transformed additive error models, were used. Model development was guided by scientific plausibility, dOFV, parameter precision, and goodness-of-fit plots, including the simulation-based visual predictive checks.

RESULTS

Pharmacokinetics of apramycin in preclinical species

The PK data from mice, rats, and guinea pigs were well-described by one-compartment models (**Table 1**). A dose-dependent k_a

Table 1 Parameter estimates of the final PK models in the preclinical species and the resulting human parameters predicted from allometric scaling

	Unit	Description	Mouse	Rat	GP	Dog	Human
fu	—	Fraction unbound	0.58	0.67	0.59	0.58	0.62
CL/F	L/hour/70 kg	Clearance	8.49	5.97	5.34	8.05	11.4
V/F	L/70 kg	Volume of distribution	6.55	8.78	11.0	17.8	43.3
k_a	$hour^{-1}$	Absorption rate constant	—	1.2	0.922	—	—
k_{a30} [a]	$hour^{-1}$	Absorption rate constant at 30 mg/kg	2.17	—		—	—
pow	—	Scaling exponent for dose-dependent Ka	-0.160	—	—	—	—
ERR	%	Residual error	49	31	25	19.3	—

GP, guinea pig.
[a]For mouse, the absorption rate at any given dose (k_a) is dose-dependent by scaling k_{a30} with *pow* as shown in Eq. 3 (see section PK/PD model development).

was significant for mice (dOFV = −49.6). The PK of apramycin in these preclinical species were not significantly different to gentamicin (in-house data). For dogs, the data supported a two-compartment model. However, because the clearance and total volume of distributions were comparable for one-compartment and two-compartment models, the PK parameters from the one-compartment model were used in the allometric scaling for consistency with the other species.

Prediction of human pharmacokinetic parameters

The CL and V values predicted by allometric scaling for a 70 kg healthy human based on total drug concentrations were 7.07 L/hour and 26.8 L, respectively (**Supplementary Figure S1**). For a typical patient with CrCL of 80 mL/minute, CL is expected to be 4.71 L/hour, assuming elimination can be scaled proportionally to CrCL. Based on the allometrically scaled PK parameters, the total concentrations of apramycin in humans were predicted to be ~ 75 mg/L at 0.5 hour after a 30 mg/kg i.v. dose infused over 0.5 hour. The predicted concentrations of apramycin were similar to those predicted using the gentamicin PK model reported in the literature[32] (**Supplementary Figure S2**).

PK/PD modeling with *in vitro* and *in vivo* efficacy data

The general structure of the PK/PD model is illustrated in **Figure 1**. Some parameter differences among the four strains were noted in the final PK/PD model (**Table 2**). For the multidrug-resistant strain EN591, the observed range of concentrations was relatively narrow in relation to its MIC and a maximum effect (E_{max}) model was not supported. Therefore, a linear relationship was applied and it was estimated to have the same slope (E_{max}/half-maximal effective concentration (EC_{50})) as for ATCC 25922. EN335 and ATCC 700336 shared the same E_{max} (slope × EC_{50}), estimated to 66.3 $hour^{-1}$. The growth rate constant was not significantly different between the strains. The delay of drug action *in vivo* was described by an effect compartment, and the EC_{50} was about 5% of what was estimated from the time-kill data. The visual predictive checks demonstrate a good fit to the time-kill and *in vivo* efficacy data (**Figure 2**).

Predicted bacterial killing curves in mice

The 24-hour bacterial killing following treatment of apramycin at 100 mg/kg q6h in mice was predicted from the developed PK/PD model (**Figure 3a**). The prediction showed a rapid killing at the start of the treatment, followed by a gradual regrowth of bacteria and eventually stasis at 24 hours. The 24-hour results are consistent with the observed data for which this regimen resulted in stasis at 24 hours for all 4 *E. coli* strains.

PK/PD targets from *in vivo* studies

When the *in vivo* efficacy data were plotted against the PK/PD indices, it was apparent that although *f*T> MIC appeared to be a good predictor of 24-hour response in the thigh infection model for apramycin, *f*AUC/MIC also showed a good correlation (**Figure 4**). The correlation between efficacy and *f*AUC/MIC increased (r^2 = 0.56 vs. 0.74) when only dosing regimens up to q6h were included. The PK/PD targets of stasis and 1-log reduction were determined to be 34.5 for stasis and 76.2 for 1-log kill (**Figure 4**).

Prediction of human efficacious dose

Dose prediction using the PK/PD model. The PK model of apramycin with predicted human PK parameters from allometric scaling was used to drive killing in the final PK/PD model. The dose that typically resulted in a bacterial count of stasis at 24 hours for the 4 strains was 30 mg/kg. This dose resulted in a similar time-kill profile as 100 mg/kg q6h in mice (**Figure 3b**). Consequently, based on this model, a 30 mg/kg q24h dose in humans is expected to be as efficient as a 100 mg/kg q6h regimen in mice at 24 hours.

PTA based on the PK/PD stasis target. Based on the PTA analysis, to reach a *f*AUC/MIC target of 30 in > 90% of the patients infected with bacteria with an MIC of 8 mg/L, apramycin doses of 30 mg/kg once daily were predicted to be sufficient (**Figure 5**). A high gentamicin dose of 7 mg/kg was predicted to cover bacteria with MICs ≤ 2 mg/L and ≤ 0.5 mg/L in 95% of the patient population based on targets of 30 and 80, respectively. Apramycin concentrations after a 30 mg/kg q24h dose are predicted to

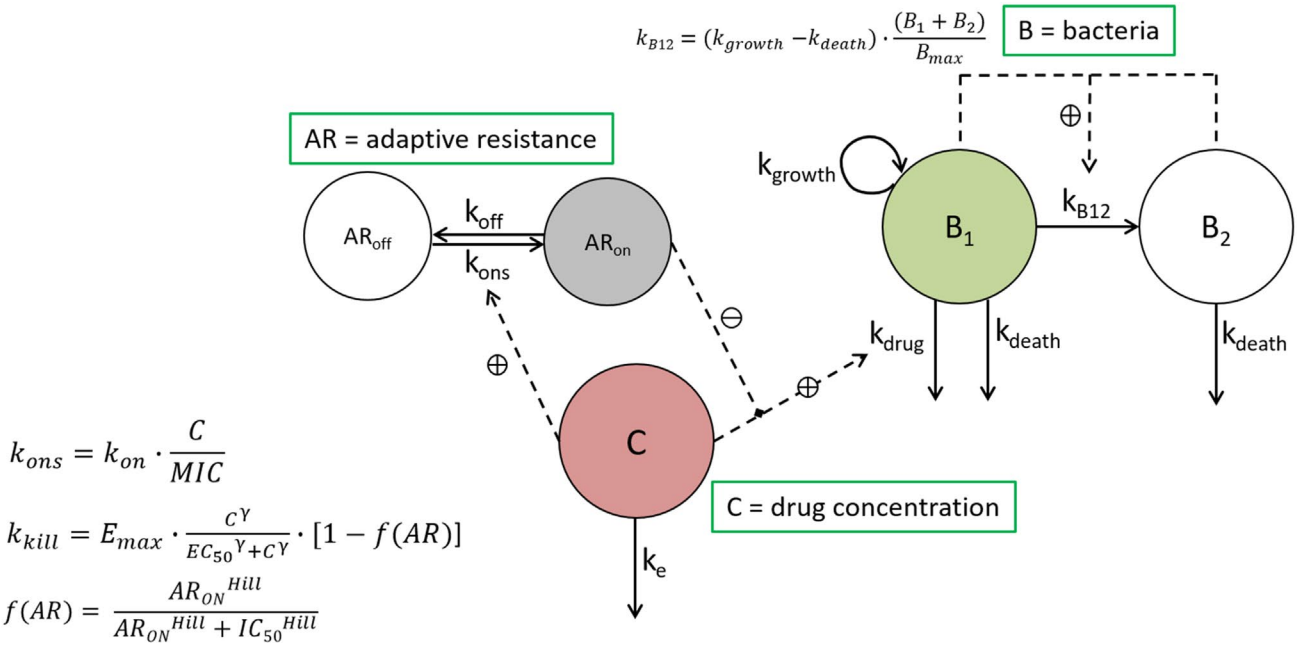

Figure 1 Schematic diagram of the pharmacokinetic/pharmacodynamic (PK/PD) model. AR, adaptive resistance; B_1, susceptible bacteria; B_2, resting bacteria; B_{max}, maximum limit of number of bacteria in the system; C, drug concentrations; E_{max}, maximum effect; k_{on}, rate constant for onset of adaptive resistance; k_{ons}, strain specific k_{on}; MIC, minimum inhibitory concentration.

be above 2 and 8 mg/L (MIC_{90} for *E. coli*) for ~ 20–24 and 8–24 hours, respectively, for 90% of patients in a population with a CrCL of 80 mL/minute.

Aminoglycoside similarity. Considering the clinical breakpoints of aminoglycosides, a four times higher daily dose of apramycin compared with the high dose gentamicin (7 mg/kg) would result in ~ 30 mg/kg. Assuming the same PK for amikacin, and given the same rationale and a clinical breakpoint of 8 mg/L, a clinical daily dose of 30 mg/kg amikacin would also scale to a 30 mg/kg dose of apramycin. Similarly, the 15 mg/kg dose of plazomicin, with an expected breakpoint of 4 mg/L,[6] would also scale to a 30 mg/kg dose of apramycin.

DISCUSSION

To determine the dose levels to be investigated in first-in-human trials, the prediction of HED from preclinical data is key. The traditional approaches for HED prediction include the classical PK/PD index approach and a PK-guided approach.[22,23,36] Here, a PK/PD model characterizing the time-courses of antibiotic-induced bacterial killing, was developed based on *in vitro* time-kill data, in combination with *in vivo* mouse infection data, to predict the human dose. This approach has the potential to advance the transition of information from preclinical to clinical studies in antibiotic drug development. Given the ability of semimechanistic PK/PD models to bridge between different types of preclinical experiments,[30,37–40] such models may provide valuable assistance in choosing a reasonable HED. Furthermore, the model-based approach applied here diminishes the need to classify the PK/PD relationship into one of the three PK/PD indices. As illustrated, the data do not always discriminate well between the PK/PD indices, implying that antibiotics may not fit into being either "time-dependent" or "concentration-dependent." Furthermore, resistant development is ignored in the classical PK/PD index approach.

In the present study, human PK was predicted by analyzing the PK data collected from four preclinical species. Multicompartment models have previously been reported for aminoglycosides in patients.[32,41] However, our data only supported one-compartment models for the preclinical species. Studies with i.v. administration and more frequent sampling for a longer duration of time would be needed to support more complex models. Nevertheless, the parameters and concentration-time profiles predicted from allometric scaling, highlighted the similarity of PK between apramycin and gentamicin. This suggests that the PK of apramycin in patients is also likely to be similar to the PK of gentamicin, and a first-in-human trial is currently in progress (ClinicalTrials.gov Identifier: NCT04105205).

The preclinical efficacy data were unique in the way that the same four strains were studied both *in vitro* and *in vivo*. The data were analyzed simultaneously to estimate PD parameters for apramycin. Regrowth at 24 hours was apparent for all strains in the time-kill experiments and explained by a function of aminoglycoside-induced adaptive resistance in the PK/PD model.[25] Adaptive resistance is known as a nonmutational phenotypic resistance, or antibiotic-induced tolerance, characterized by its transient nature.[29,42] In the present study, a simpler empirical model with only bacterial growth and killing was not able to adequately describe the data and the exposure-response relationship. In the model, the differences in the time-kill curves between strains could be explained by the differences in maximum growing capacity, drug susceptibility, and rate of resistance development. The different drug effects

Table 2 Parameter estimates and RSEs of the final PK/PD model

Parameter	Unit	Description	Strain[a]	Value	% RSE
k_{g_vitro}	$hour^{-1}$	Bacteria growth rate constant —*in vitro*	All	2.16	3.5
B_{max}	Log CFU/mL	Maximum bacterial count limit —*in vitro*	ATCC 25922[a]	9.38	3.5
			ATCC 25922[b]	8.47	2.1
			EN335	9.47	2
			EN591	9.43	1.4
			ATCC 700336	9.48	1
		Maximum bacterial count limit —*in vivo*	ATCC 25922	8.97	Fix
			EN335	8.59	Fix
			EN591	7.76	Fix
			ATCC 700336	7.56	Fix
Slope	L/mg/hour	Ratio of maximum killing rate constant (E_{max}) and EC_{50}	ATCC 25922 EN591	0.645	9.1
			EN335	0.425[b]	NA
			ATCC 700336	0.796	9.4
EC_{50}	mg/L	Drug concentration needed to achieve 50% of E_{max}	ATCC 25922	123	17.1
			EN335	156	12.7
			EN591	268	13.7
			ATCC 700336	83.3	14.9
k_{on}	$hour^{-1}$	Rate constant for onset of adaptive resistance, scaled by drug concentration and MIC	All	0.555	10.5
k_{off}	$hour^{-1}$	Rate constant for reversal of adaptive resistance	All	0.005	Fix
Hill	—	Sigmoidicity factor for adaptive resistance	All	20	Fix
IC_{50}	mg/L	Fraction needed to achieve 50% of the maximum concentration-driven resistance	ATCC 25922 ATCC 700336	0.944	0.8
			EN335	0.958	0.8
			EN591	0.905	0.6
k_{g_vivo}	$hour^{-1}$	Bacteria growth rate constant —*in vivo*	All	0.944	5.1
T_{delay}	Hour	Growth delay *in vivo*	All	0.849	16.1
Beta (β)	—	Sigmoidicity factor for growth delay *in vivo*	All	5	Fix
k_{eff}	$hour^{-1}$	Rate constant for effect compartment *in vivo*	All	0.313	24.2
EC_{shift}	—	Shift factor for EC_{50} *in vivo*[c]	All	0.0537	6.4
ERR	%	Residual error—*in vitro*	ATCC 25922a	118.3	13.5
			ATCC 25922b	140.4	12.4
			EN335	74.5	30.4
			EN591	66.0	13.9
			ATCC 700336	80.4	17.0
		Residual error—*in vivo*	All	84.9	3.6

APR, apramycin; B_{max}, maximum number of bacteria in the system; CFU, colony-forming unit; EC_{50}, half-maximal effective concentration; E_{max}, maximum effect; ERR, residual error; IC_{50}, half-maximal inhibitory concentration; MIC, minimum inhibitory concentration; NA, not applicable.
[a]ATCC 25922 a and b denote data from different sets of experiments. [b]The slope parameter of EN335 was computed as: $Slope_{EN335} = Slope_{EN1085} \times EC_{50_EN1085}/EC_{50_EN335}$. [c]$EC_{50}$ *in vivo* was parameterized as a shift from EC_{50} *in vitro*: $EC_{50_vivo} = EC_{50_vitro} \times EC_{shift}$.

observed *in vivo* was described by a delayed drug action that could be due to drug distribution from plasma to the site of action and an increase in drug potency, as suggested by the lower EC_{50} parameter. The higher potency *in vivo* could potentially indicate higher free drug concentrations at the target site of action than what would be expected from the effect compartment model, or that the environment in the mouse thigh increases the susceptibility of the bacteria compared with *in vitro* conditions.

In the dose fractionation studies, apramycin showed lower CFU reduction following q12h and q24h dosing compared with the same total dose fractionated to more frequent dosing. The USCAST evaluation on aminoglycosides[36] was based on gentamicin, tobramycin, and amikacin with dosing intervals of 6 hours and shorter, because schedules of q12h or q24h were not deemed suitable for determining PK/PD index correlations of aminoglycosides given the short half-lives in relation to the dosing interval.

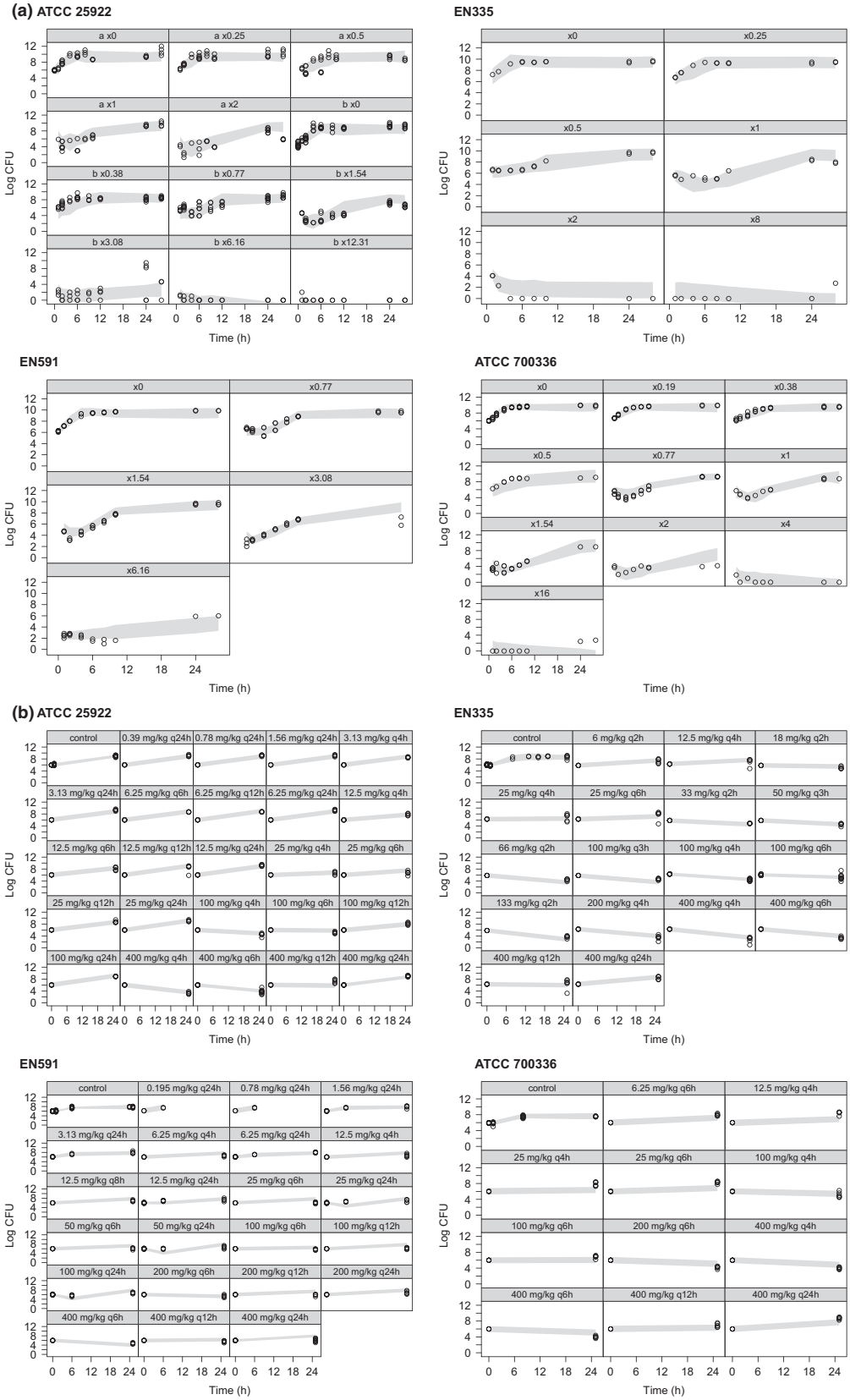

Figure 2 Model evaluation of the final model. Visual predictive checks are illustrated for both the (**a**) *in vitro* time-kill and (**b**) in vivo efficacy studies for the four *Escherichia coli* strains. The plots show the observed bacterial count data (Log CFU) of ATCC 25922, ATCC 700336, EN335 and EN591 at different apramycin concentrations (xMIC) for *in vitro* time-kill studies and different dosing intervals for *in vivo* efficacy studies with model predictions as 95% confidence intervals of the predicted medians (shaded). Data below the limit of detection are plotted as 0. CFU, colony-forming unit.

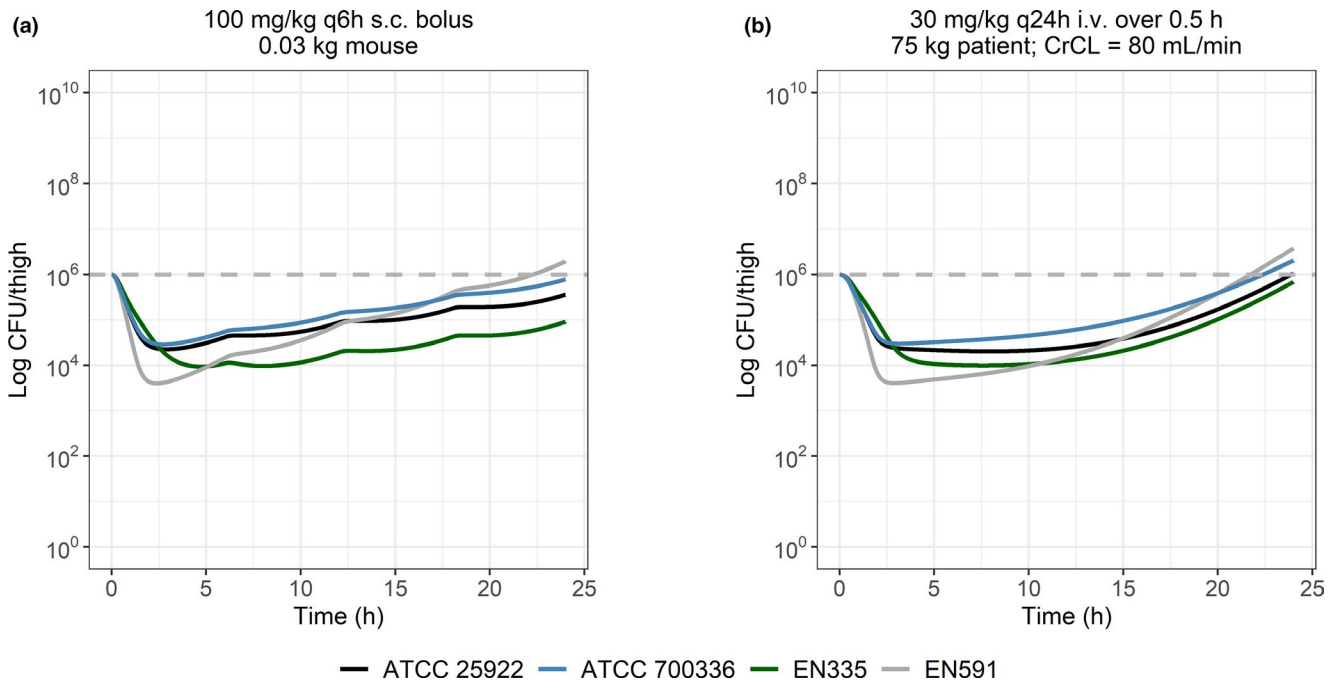

Figure 3 Model-predicted bacterial killing *in vivo*. Predictions are shown for AATCC 25922, ATCC 700336, EN335 and EN591 in mice (**a**) and in patients (**b**) following 24-hour treatment of apramycin at 100 mg/kg q6h (mice) and 30 mg/kg q24h (patients) based on the final PK/PD model. CFU, colony-forming unit; CrCL, creatinine clearance; PK/PD, pharmacokinetic/pharmacodynamic.

It has previously been shown that the best correlated index shifts toward *f*AUC/MIC from *f*T>MIC as the half-life of the drug increases.[37,43] In the present study, the correlation to the *f*AUC/MIC index improved when the longer dosing intervals (q12h and q24h) were removed from the analysis (data not shown). Previously, Craig noted that *f*AUC/MIC was the major PK/PD index correlating with efficacy for aminoglycosides when dosing regimens varied from 1 to 6 hours in mice.[44] Indeed, a short elimination half-life of the antibiotic in relation to the dosing interval favors *f*T>MIC. In humans, who have longer elimination half-lives, *f*AUC/MIC is more often the better index, as suggested for aminoglycosides. Hence, although the R^2 values for *f*T>MIC and *f*AUC/MIC were comparable, the latter was chosen as the most appropriate target for PTA simulations of human dosing. The PK/PD targets determined in this study are comparable to those stated by USCAST.[36] For complicated urinary tract infections, where the

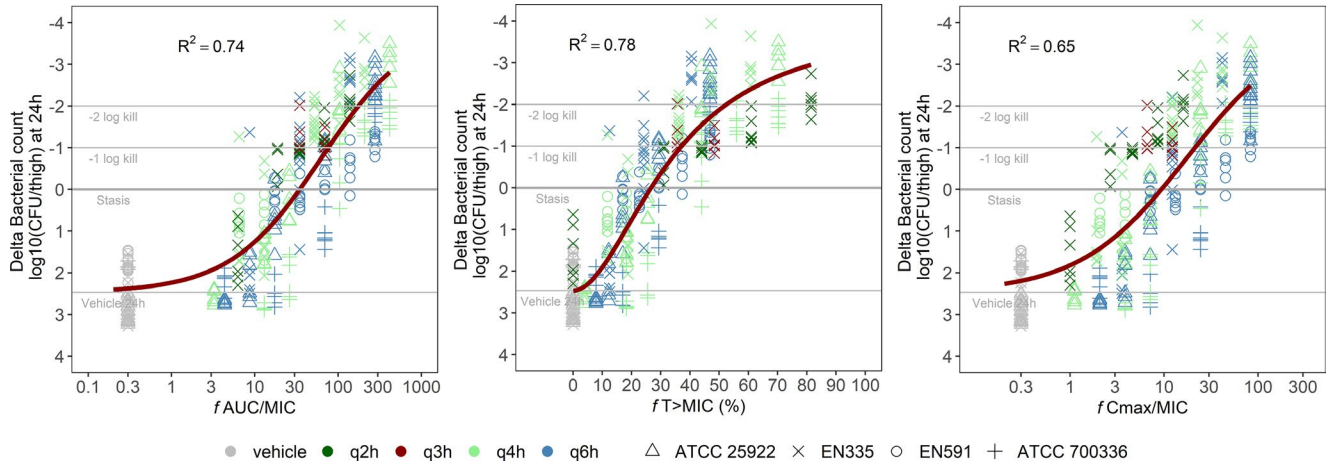

Figure 4 CFU response at 24 hours vs. PK/PD indices data in the mouse thigh infection model for ATCC 25922, ATCC 700336, EN335, and EN591 including dosing regimens up to every 6 hours. Baseline with no effect was set to the mean value of the observed data from the control groups. Maximum response was fixed to four log-kill from start of treatment. The MIC was set to 8 mg/L for EN335 and 4 mg/L for all other strains. CFU, colony-forming unit; C_{max}, peak plasma concentration; fAUC, area under the unbound drug concentration-time curve; MIC, minimum inhibitory concentration.

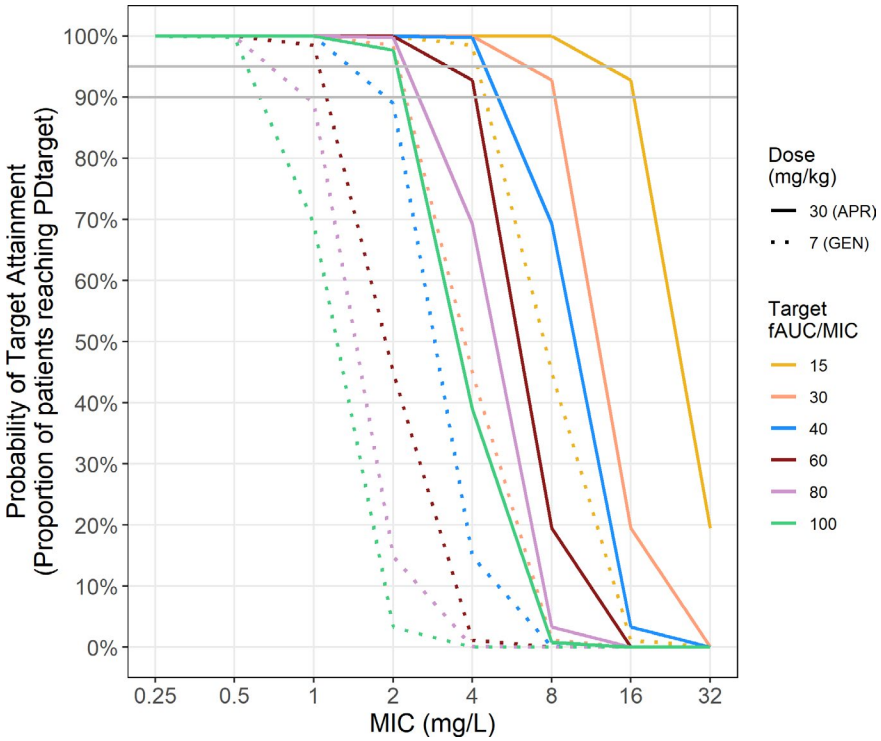

Figure 5 Probability of target attainment (PTA) in patients with a creatinine clearance (CrCL) of 80 mL/minute using the PopPK model of gentamicin considering the fraction unbound (f_u) of the drugs (0.62 for APR and 0.83 for GEN). APR, apramycin; fAUC, area under the unbound drug concentration-time curve; GEN, gentamicin; MIC, minimum inhibitory concentration; PD, pharmacodynamic; PopPK, population pharmacokinetic.

drug concentrates at the effect site, the stasis end point in the thigh model has been suggested to be an appropriate surrogate target for efficacy.[36]

The PK/PD modeling approach resulted in the same dose prediction as the PK/PD index approach. However, the modeling approach provides more information because all data can be considered to further the knowledge of bacterial growth, the concentration-effect relationship of the antibiotic, and the rate and concentration-dependence of resistance development. Furthermore, the PK/PD model developed can be used to explore *in vivo* bacterial dynamics at various time points following different dosing regimens. Considering the potential differences in growth rates and drug susceptibility, the model can also be applied to predict bacterial dynamics of untested strains with other fitnesses and MICs. Given that the PD measurements in animals are terminal, *in vivo* bacterial counts after apramycin dosing were only available at two time points. The PK/PD model allowed the prediction of the time course of bacterial dynamics by integrating the sparse *in vivo* data with the richer *in vitro* data.

The results of the three approaches investigated in the present study are comparable and all support a daily dose of 30 mg/kg for patients with a typical CrCL of 80 mL/minute. The comparability of the predictions indicates that the PK/PD modeling approach can be a suitable method for dose prediction. In addition, this modeling approach can serve as an efficient method to integrate all available *in vitro* and *in vivo* data, both PK and efficacy, in a mechanistic manner to increase the predictive capacity and allow exploration of different scenarios. Hence, the modeling approach can maximize the information obtained from the various studies in a way not possible with classical methods. In particular, the modeling approach can take into account the mechanism of action of the drug and the emergence of resistance, and hence project potential impact of these factors. Therefore, it can be particularly valuable for the evaluation of combination therapies, where drugs with different PK properties and actions are used concurrently, because the classical methods are only applicable to monotherapy. Model-informed drug development is likely to play an increasingly valuable role in the development of new treatments.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

S.N.H. is a co-founder of Juvabis AG, a startup biotech company with an interest in aminoglycoside therapeutics. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

T.S. wrote the manuscript. T.S., L.E.F., and S.N.H. designed the research. J.H., E.L., M.B., S.G., O.E., S.C., P.G., A.P., M.T., M.U., D.Z., C.V.L., and D.H. performed the research. T.S., L.E.F., and S.N.H. analyzed the data. S.N.H. contributed reagents.

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