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An integrative and translational PK/PD modelling approach to explore the combined effect of polymyxin B and minocycline against *Klebsiella pneumoniae*

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ABSTRACT

Objectives: To expand a translational pharmacokinetic–pharmacodynamic (PK/PD) modelling approach for assessing the combined effect of polymyxin B and minocycline against *Klebsiella pneumoniae*.
Methods: A PK/PD model developed based on *in vitro* static time-kill experiments of one strain (ARU613) was first translated to characterize that of a more susceptible strain (ARU705), and thereafter to dynamic time-kill experiments (both strains) and to a murine thigh infection model (ARU705 only). The PK/PD model was updated stepwise using accumulated data. Predictions of bacterial killing in humans were performed.
Results: The same model structure could be used in each translational step, with parameters being re-estimated. Dynamic data were well predicted by static-data-based models. The *in vitro*/*in vivo* differences were primarily quantified as a change in polymyxin B effect: a lower killing rate constant *in vivo* compared with *in vitro* (concentration of 3 mg/L corresponds to 0.05/h and 57/h, respectively), and a slower adaptive resistance rate (the constant *in vivo* was 2.5% of that *in vitro*). There was no significant difference in polymyxin B–minocycline interaction functions. Predictions based on both *in vitro* and *in vivo* parameters indicated that the combination has a greater-than-monotherapy antibacterial effect in humans, forecasting a reduction of approximately 5 and 2 $\log_{10}$ colony-forming units/mL at 24 h, respectively, under combined therapy, while the maximum bacterial load was reached in monotherapy.
Conclusions: This study demonstrated the utility of the PK/PD modelling approach to understand translation of antibiotic effects across experimental systems, and showed a promising antibacterial effect of polymyxin B and minocycline in combination against *K. pneumoniae*.

1. Introduction

Combination therapies including two or more antibiotics are used as a last-resort strategy [1] in severe infections caused by multi-drug-resistant Gram-negative bacteria, such as carbapenem-resistant *Klebsiella pneumoniae*. However, evaluating the efficacy of combination therapies in clinical studies is complex, and evidence remains scarce [2,3]. Model-based translational approaches, including preclinical *in vitro* and *in vivo* data in combination with semi-mechanistic pharmacokinetic–pharmacodynamic (PK/PD) modelling and simulation, may be used to support the selection of promising combination therapies to test in clinical studies [4]. PK/PD models enable quantitative description of the full time-course of the interaction between bacteria and antibiotics, including the dynamic changes of effective antibiotic concentrations (PK) as well as the antibiotic-induced suppression of bacterial growth, killing of bacteria, and/or development of resistance (PD). Moreover, PK/PD mod-

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els can be employed to simulate and predict the drug effect in scenarios beyond the experimental settings used for model building.

As a continuous and iterative learn-and-confirm process, model-based translational approaches have the capacity to preserve and integrate the information gathered at each development stage from preclinical to clinical by updating the PK/PD models progressively, and thus enhance their predictability of the antibiotic effect and the doses that will have the greatest benefit for patients. Such translational approaches have been applied successfully to the development of apramycin, leading to the suggestion of a human efficacious dose of 30 mg/kg once daily [5,6]. In these studies on apramycin, semi-mechanistic PK/PD models were applied stepwise to make use of all PK and PD data from *in vitro* and *in vivo* studies. A similar translational approach may also be used in the development of antibiotic combination therapies.

The combination of polymyxin B and minocycline has been found to be effective *in vitro*, and of potential clinical interest against multi-drug-resistant *K. pneumoniae* [7]. In a previous study, the authors developed a semi-mechanistic PK/PD model based on *in vitro* static time-kill experiments conducted with a clinical carbapenemase-producing *K. pneumoniae* strain (ARU613) resistant to polymyxin B and minocycline [8]. The authors had previously demonstrated the translational capacity of this model in (i) predicting *in vitro* dynamic time-kill data for ARU613; and (ii) extrapolating to static time-kill data of a more susceptible strain (ARU705), followed by predicting dynamic time-kill data of this strain [9]. The current study aimed to expand this translational approach by integrating all available *in vitro* and *in vivo* data from the neutropenic murine thigh infection model for the estimation of model parameters that fit all included data.

To mimic different stages of the translational approach in a drug development setting, where data and knowledge accumulate over time, translation was conducted stepwise from *in vitro* static to *in vitro* dynamic to *in vivo* murine conditions. The PK of polymyxin B and minocycline in mice, used to drive the PK/PD model, was characterized by developing compartmental models using data collected from several PK studies conducted in murine infection models. The in-silico PK/PD models derived from each translational stage – that is, (i) only *in vitro* static data available; (ii) *in vitro* static and *in vitro* dynamic data available; and (iii) *in vitro* static, *in vitro* dynamic and *in vivo* data available – were used to predict bacterial killing in humans under commonly prescribed clinical dosing regimens as monotherapy and in combination, and thus to evaluate the potential of combining polymyxin B and minocycline in the treatment of *K. pneumoniae* infections.

2. Materials and methods

2.1. Strains

The two strains used in this study were clinical carbapenemase-producing *K. pneumoniae* isolates provided by the Public Health Agency of Sweden (ARU613, producing OXA-48) and by Erasmus MC in Rotterdam, Netherlands (ARU705, also called *K. pneumoniae* 104 [10], producing KPC-3). The minimum inhibitory concentrations (MICs) for polymyxin B were 0.5 and 8 mg/L for ARU705 and ARU613, respectively; corresponding figures for minocycline were 1 and 8 mg/L, respectively [9]. The more resistant strain (ARU613) was only investigated *in vitro* as no or limited effect would be expected *in vivo* at tolerable doses. ARU705 was chosen due to its proven *in vivo* virulence, and was used both *in vitro* and *in vivo*. More details about phenotypic and genetic characterization of these two strains can be found elsewhere [9].

2.2. in vitro *static and dynamic time-kill experiments*

in vitro static and dynamic (in-house kinetic model) time-kill experiments were carried out at Uppsala University, Sweden and have been reported previously [8,9]. In short, the bacterial start inoculum was approximately 5×10^6 colony-forming units (CFU)/mL, with a working volume of 3 and 100 mL for static and dynamic experiments, respectively. Antibiotics were introduced at a fixed concentration or according to designs mimicking the expected free plasma concentrations in critically ill patients (details in Table 1; same dosages and PK profiles described in Section 2.5). Samples for determination of CFU were collected repeatedly over 28 h (static experiments) or 72 h (dynamic experiments). The lower limit of detection (LOD) was 1 $\log_{10}$ CFU/mL. For dynamic experiments, an upper limit of quantification (LOQ) of 8 $\log_{10}$ CFU/mL was applied, due to clogging of the filter. All experiments were performed at least in duplicate.

2.3. in vivo *PK and PK/PD studies*

in vivo studies were carried out at Erasmus Laboratory Animal Science Centre in Rotterdam, The Netherlands, in accordance with EU Animal Directive 2010/63/EU 2010 (IRN 2019-0018), and were approved by the institutional animal welfare body. The experimental settings were similar to those reported previously for polymyxin B in monotherapy [10]. In short, outbred female CD-1 mice (weighing approximately 25 g) were rendered neutropenic (intraperitoneal cyclophosphamide 150 and 100 mg/kg on day 4 and day 1 before infection, respectively) before the experiments, and inoculated with 0.05 mL culture (approximately 5.0×10^7 CFU/mL) to reach approximately 2.5×10^6 bacteria/thigh. Subcutaneous antibiotic administration was initiated 2 h after inoculation.

Antibiotic dosages used for PK and PK/PD studies are listed in Table 1. For the PK studies, which were carried out separately from the PK/PD studies in murine infection models, plasma concentrations were sampled (two mice per sample point) through orbital sinus bleeding from 5 min to 8 h (for minocycline) or 12 h (for polymyxin B) post dose. The polymyxin B PK studies were conducted on two occasions, where Occasion 1 has been reported previously [10] and Occasion 2 included data at additional time points. Antibiotic concentrations were determined by validated liquid chromatography tandem mass spectrometry ([10] and Supplementary Text S1). The lower LOQ was 10 ng/mL for minocycline, and 200 ng/mL and 100 ng/mL, on Occasions 1 and 2 respectively, for polymyxin B. The PK/PD studies were conducted in the thigh infection model (intramuscular infection of ARU705 in hind legs, two mice per dose regimen), the mice were euthanized at 0 h (i.e. 2 h after infection, as infection control) or at 24 h post first dose, unless earlier termination was necessary according to the animal welfare regulations. The polymyxin B monotherapy studies have been reported previously [10]. Minocycline monotherapy and the combination therapy studies will be reported in a separate article. In short, excised thighs were homogenized in 2 mL pre-cooled phosphate buffered serum using a T25 ULTRA-TURRAX (IKA-Werke GmbH & Co, Staufen, Germany). From these homogenates, 10-fold dilution series were prepared and plated (three drops of 10 µL per dilution, 200 µL of undiluted homogenate) for CFU counts and reported as CFU/thigh (lower LOQ was 10 CFU/thigh).

2.4. PK/PD model building and translation

2.4.1. Murine PK models

One- or two-compartment PK models; (parallel) linear and/or non-linear (saturable Michaelis–Menten kinetics) clearance; (parallel) dose-dependent saturable and first-order absorption rate constant were compared. Dose-dependent relative bioavailability was

Table 1
Doses/concentrations used in the *in vitro* and *in vivo* experiments.

Pharmacokinetic studies[a]		
PMB	0.5–64 mg/kg (single doses, increasing two-fold)[b]	
MIN	1–64 mg/kg (single doses, increasing four-fold)	
Pharmacokinetic–pharmacodynamic studies		
	Static time-kill experiments (concentrations increasing two-fold for single drug)	
Strain	**ARU705**[c]	**ARU613**[d]
PMB	0.0625–64 mg/L (i.e. 0.125–128 x MIC, n=11)	1–128 mg/L (i.e. 0.125–16 x MIC, n=8)
MIN	0.25–256 mg/L (i.e. 0.25–256 x MIC, n=11)	1.5–768 mg/L (i.e. ~0.19–96 x MIC, n=10)
COMB	PMB 0.25–1 mg/L + MIN 0.5–16 mg/L (n=9)	PMB 1–8 mg/L + MIN 1.5–12 mg/L (n=9)
	Dynamic time-kill experiments (same design for both ARU613 and ARU705)[c]	
PMB	C_{max} 5.0 mg/L after first dosing and around 4.5 mg/L in the following peaks, C_{min} around 1.1 mg/L	
MIN	C_{max} 3.8 mg/L after first dosing and around 4.1 mg/L in the following peaks, C_{min} around 1.8 mg/L	
COMB	Combination of the above	
	Murine thigh infection model (doses increasing two-fold for both single drug and combination)	
Strain	**ARU705**	**ARU613**
PMB	4–128 mg/kg q6h, 8–256 mg/kg q12h, 256–512 q24h (n=14)[b]	NA
MIN	4–128 mg/kg q2h, 4–128 mg/kg q4h, 16–512 mg/kg q6h (n=18)	NA
COMB	MIN 4–64 mg/kg q2h + PMB 8–16 mg/kg q6h, MIN 32–64 mg/kg q6h + PMB 8–16 mg/kg q6h and q12h (n=14)	NA

PMB, polymyxin B; MIN, minocycline; COMB, combination; C_{max}, peak drug concentration: C_{min}, trough drug concentration; qnh, every n hours; NA: not applicable.
[a] Pharmacokinetic studies are from infected mice. For polymyxin B, mice were infected in either thigh (*Klebsiella pneumoniae* ATCC43816 and *Escherichia coli* ATCC25922) or lung (*K. pneumoniae* ATCC 43816). For minocycline, mice were infected in lung (*Staphylococcus aureus* ATCC29213).
[b] Part of the PMB pharmacokinetic data (referred to as Occasion 1 in the text) and PMB data in the murine infection model have been published previously [10].
[c] Static time-kill data for ARU705 and dynamic time-kill data from both ARU613 and ARU705 have been published previously [9]. The targeted human pharmacokinetic profiles in the dynamic time-kill experiments are described in Supplementary Text S4 [models Polymyxin B and Minocycline (Welling)]. The targeted experimental profiles were simplified to a bolus injection rather than a 1-h infusion to mimic human pharmacokinetic profiles, which are summarized in the table. This, together with other details of the dynamic model, including the setting of the half-lives, has been described previously [9].
[d] Static time-kill data for ARU613 have been published previously [8].

tested. As PK studies were performed in satellite animals and the typical trends were of interest, no interindividual variability in the parameters was considered. Data from the two polymyxin B PK studies were analysed together. The data from the 8 mg/kg dose group from Occasion 1, which had unexpectedly low concentrations (even lower than the concentrations from the 4 mg/kg dose group from Occasion 1 as shown in [10] and lower than the concentrations from the 8 mg/kg dose group from Occasion 2), were excluded from model building. The exclusion was supported by a sensitivity analysis, which showed that including these data in model building would result in slightly underpredicted concentration profiles at all time points, especially for peak concentrations.

2.4.2. Model-based translation of PK/PD studies

An overview of the translation approach is summarized in Fig. 1. The model built on *in vitro* static time-kill data for ARU613 [8] was the starting model. Schematic structure and equations of the model are shown in Supplementary Text S2. This model structure was applied consistently across all steps, including extrapolation from ARU613 to ARU705 and transitions from *in vitro* static to dynamic (for both ARU613 and ARU705) to *in vivo* murine (for ARU705) conditions. In each step, the PK/PD model from the previous step was used to examine the model's predictive capacity by comparing model predictions (without parameter re-estimation) with the newly collected observations, followed by model updating (with parameter re-estimation) with all cumulatively available data.

Polymyxin B labware binding [11] was considered in modelling static time-kill data (Supplementary Table S1). For the dynamic data, the binding was corrected for in the experimental design using 1.67-fold target [9] concentrations, thus no further correction in the modelling was needed. For ARU613, the capacity of the model built on static data to predict dynamic data without re-estimation of parameters has been reported previously [9], while here model parameters were re-estimated in a simultaneous fit to both static and dynamic data. For ARU705, no modelling results presented here have been reported previously as this study re-estimated all parameters to describe ARU705 *in vitro* static time-kill data, while previous work [9] only re-estimated a limited number of parameters (n=5) when extrapolating from ARU613. This was followed by re-predicting dynamic data and then updating the model parameters with combined *in vitro* static and dynamic data. Thereafter, the updated *in vitro* PK/PD model was applied to predict CFU profiles of the murine thigh infection model, and subsequently updated using combined *in vitro* and *in vivo* data. At this stage, parameters could differ for the *in vitro* and *in vivo* settings if indicated by the data. The unbound plasma concentration–time profiles that drove the PD part of the *in vivo* PK/PD model were predicted from the developed murine PK models and the unbound fraction (fu). For polymyxin B, fu in murine plasma was set at 20% [10], and for minocycline, non-linear fu, as shown below, was used:

$$\ln\left(f\mathrm{u}, mice, \%\right) = 5.48 - 0.331 \times \ln\left(Ct,\ \mu g/L\right),\ \mathrm{R2} = 0.95$$

where Ct is total plasma concentration in μg/L, and fu, *mice* is fu in murine plasma as a percentage. The equation was derived from published protein binding data [12] which described an atypical concentration dependency with lower *fu* at higher minocycline concentrations.

2.4.3. Modelling tools

PK/PD modelling was performed in NONMEM v. 7.4 or higher. Model discrimination was based on a combined assessment of statistical significance (P<0.001, dOFV>10.83 and df=1), improvement in fit according to diagnostic [e.g. goodness-of-fit (GOF) and visual predictive checks (VPC) [13]] plots and mechanistic plausibility.

2.5. Prediction of drug effect in humans with ARU705 PK/PD models

PK/PD models were derived for ARU705 describing (i) static time-kill data; (ii) static and dynamic time-kill data; and (iii) static and dynamic time-kill data as well as *in vivo* thigh data. These models were used to predict the effect in patients following a loading + maintenance dose of polymyxin B 2.5 mg/kg + 1.5 mg/kg every 12 h (q12h); minocycline 400 mg + 200 mg q12h. All doses mimicked a 1h intravenous infusion. The adopted PK profiles for patients were the same as used previosuly [8], and also from the latest published minocycline population PK model [14]. Details of

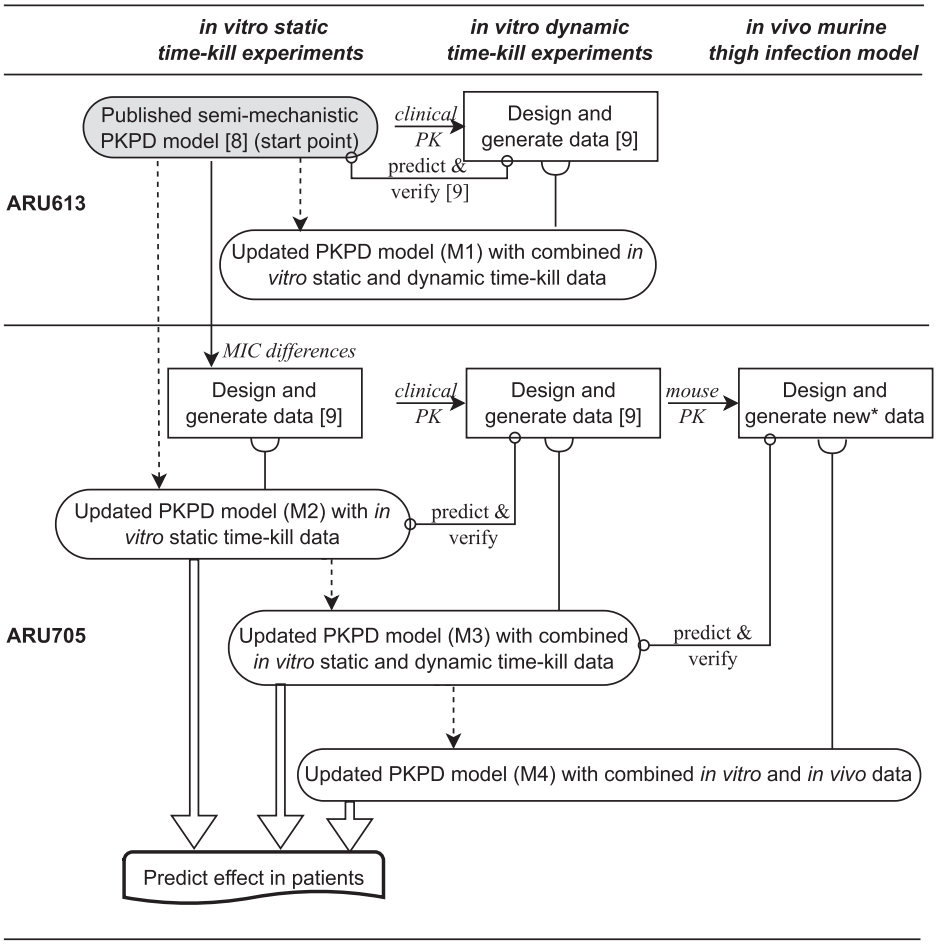

Fig. 1. Flow chart of the translational pharmacokinetic–pharmacodynamic (PK/PD) modelling and simulation studies for ARU613 and ARU705 across different experimental settings. The published semi-mechanistic PK/PD model for ARU613 from the static time-kill experiments [8] (grey oval shape) served as the starting point. This model (and the following updated models) was used to design new experimental studies for both ARU613 and ARU705 by considering the differences in minimum inhibitory concentrations of the strains, clinical PK profiles for *in vitro* dynamic experiments, and murine PK profiles for *in vivo* experiments. Datasets used for modelling have either been published previously for other purposes [9,10] or were generated specifically for this study. (* indicates that all *in vivo* data were generated in this study except for polymyxin B monotherapy data [10].) In each translational step, the existing model was used to predict time-kill profiles under new conditions to facilitate the design and evaluate the current model based on the generated data (connection line with open circles). The 'predict and verify' step for ARU613 has been reported previously [9]. In the final step, the PK/PD model was updated by combining all available data for ARU705 (dashed arrow and y-shape connection line pointing to plain oval shape). The same model structure was used in all steps, but parameters were re-estimated. These models were named M1–M4, and the corresponding parameter estimates are reported in Table 3. The three PK/PD models developed for ARU705 were used to predict the bacterial killing effects of the antibiotics in patients who were administered clinically recommended doses (hollow arrows).

these adopted PK models and *fu* values are given in Supplementary Text S4. It was assumed that the starting bacterial load in each patient was 6.8 $\log_{10}$ CFU/mL, the same value as used previously [8].

To account for parameter uncertainty, standard errors of the PK/PD model parameters (M2–M4 in Section 3.3) were considered in the predictions, in addition to interindividual variability of the population PK parameters (Supplementary Text S4). The predictions were conducted in mrgsolve Version 1.0.6 in R [15].

3. Results

3.1. Early termination *in* in vivo *studies*

In the *in vivo* PK/PD experiments, it was noticed that high doses of polymyxin B (>64 mg/kg per administration) were not tolerated. Minocycline had both solubility and tolerability issues at high dose levels. As these doses were in suspension, not a solution, at the time of injection, this may have caused the local toxicity that was observed at the injection site in the neck, resulting in incomplete spread of minocycline throughout the body. Seven mice in the PK/PD studies receiving high minocycline doses reached the humane endpoint before the end of the study. In addition, in the control groups and under minocycline monotherapy at low doses, some mice reached the humane endpoint before the end of the study. The time of euthanasia and CFU counts from these mice were included in the PK/PD modelling dataset.

3.2. PK models in mice

Murine PK profiles were best described by a two-compartment model with saturable clearance for polymyxin B, and a one-compartment model with linear clearance for minocycline. Model parameters are listed in Table 2. The model fit to the observed concentrations, as assessed by VPCs, is shown in Fig. 2. Their predicted concentration–time profiles, which drove the *in vivo* part of the PK/PD models, are shown in Supplementary Fig. S1.

3.3. Model-based translation of PK/PD studies

For ARU613, the model parameter estimates based on combined static and dynamic data were similar to those estimated previously

Table 2
Parameter estimates for mouse pharmacokinetic (PK) models.

PK parameter	Description	Estimation (RSE)
Polymyxin B		
$k_{a-1mg/kg}$ (/h)[a]	k_a at a dose of 1 mg/kg	2.10 (21%)
Abs (-)*	Exponent of the power absorption function	-0.348 (10%)
V_c (L/kg)	Central compartmental volume	0.405 (23%)
V_{max} (mg/h/kg)	Maximum rate of saturable elimination	4.62 (5.9%)
K_m (mg/L)	Plasma concentration at ½ V_{max}	6.85 (9.0%)
V_p (L/kg)	Peripheral compartmental volume	0.730 (9.9%)
Q (L/h/kg)	Inter-compartmental clearance	0.213 (11%)
Additive residual error on log-transformed data (SD, log mg/L)		0.315 (6.3%)
Minocycline		
F_{50} (mg/kg)[b]	Log dose resulting in 50% relative bioavailability	2.09 (11%)
k_a (/h)	First-order absorption rate constant	3.20 (11%)
CL (L/h/kg)	Clearance	0.563 (6.3%)
V (L/kg)	Volume	2.25 (6.1%)
Additive residual error on log-transformed data (SD, log mg/L)		0.229 (7.6%)

RSE, relative standard error.

[a] First-order absorption rate constant (k_a) = $k_{a\_1mg/kg} \times (dose)^{Abs}$; dose in mg/kg; assume bioavailability (F) = 1.

[b] Bioavailability (F) = $1 - \frac{LN(dose)}{F50 + LN(dose)}$; dose in mg/kg; assume F = 1 at lowest dose in the study (1 mg/kg). For the highest dose of 64 mg/kg in this study, the corresponding F is 33%.

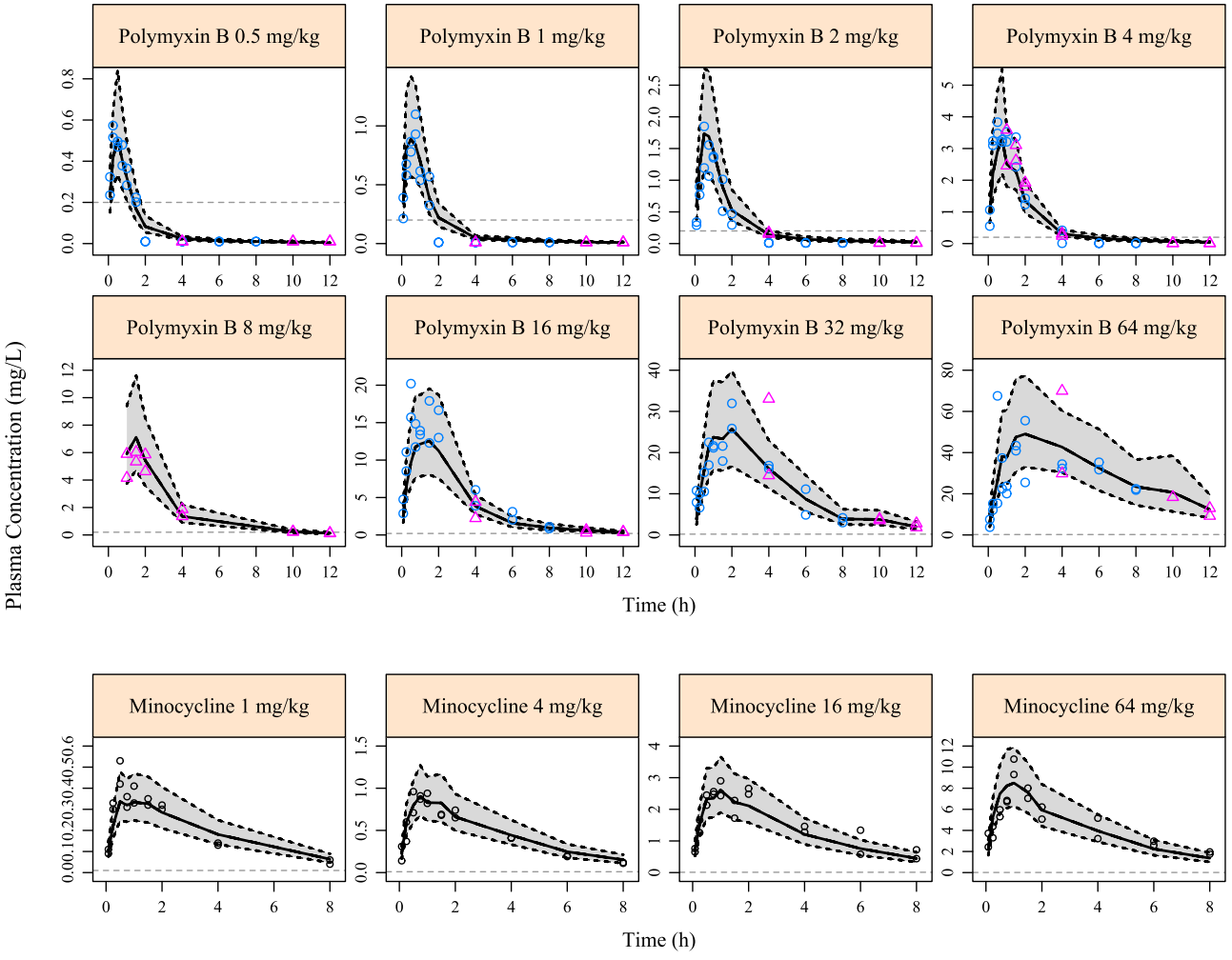

Fig. 2. Visual predictive checks for the pharmacokinetic models for polymyxin B and minocycline, stratified by dose. The observed plasma concentrations [minocycline: open circles; polymyxin B – Occasion 1 (published [10]): blue circles; Occasion 2 (newly collected), red triangles], as well as the median (solid line) and its 95% confidence interval (grey shading) of the simulated data (n=1000) are shown. The limit of quantification is shown as a horizontal dashed line.

from static data alone (Table 3, M1 vs. [8]). VPC plots (Supplementary Fig. S2) showed that the combined model fits both types of data.

The same model structure could describe the data for ARU705, although parameter estimates differed (Table 3, M2), as expected given the differences in MIC values (i.e. polymyxin B 0.5 mg/L and 8 mg/L and minocycline 1 mg/L and 8 mg/L for ARU705 and ARU613, respectively). However, the lag time before bacterial transit from susceptible to resting state was no longer needed and was omitted. The predicted dynamic profiles (Supplementary Fig. S3) mirrored the observations. However, re-estimation based on combined dynamic and static data (Table 3, M3) improved the model fit further, especially for the effect of minocycline monotherapy.

When this updated model was used to predict *in vivo* drug effects, overall, the predictions were reasonable for minocycline given the different experimental systems. However, the drug effect was overpredicted for polymyxin B monotherapy at high dosages and for all drug combination therapies (Supplementary Fig. S4). The overprediction was also obvious when the parameters representing drug-free bacterial growth kinetics (i.e. k_{growth} and Bmax) were fixed to values estimated from *in vivo* growth control data (Supplementary Fig. S5). These misfits were resolved by re-estimating parameters using a combined *in vitro* and *in vivo* data set, and allowing a selection of parameters have different parameter values for *in vitro* and *in vivo* data (Table 3, M4), reflecting the discrepancies between the two systems. k_{growth} and Bmax were kept fixed during the process to avoid potential misinterpretation of the drug effects caused by compensating the fit to the growth control data. The *in vivo* polymyxin B monotherapy killing rate constant (k_{drug}) was re-parameterized by a sigmoid Emax function, and the adaptive resistance development rate constant (k_{on}) was re-estimated. The rate constant for adaptive resistance disappearance (k_{off}) was kept fixed at 0 due to difficulties in estimating this parameter, indicating that the level of resistance increased over time in the scope of the experimental settings. Both k_{drug} and k_{on} were lower than their values in the *in vitro* settings. Polymyxin B k_{drug} *in vivo* was 0.05/h at 3 mg/L and reached the maximum value of 3.7/h at >5 mg/L. The estimated *in vitro* k_{drug} was 57 and 115/h at 3 and 5 mg/L, respectively, and >4/h at concentrations >0.5 mg/L. Polymyxin B k_{on} for *in vivo* data was 2.5% of the value estimated from *in vitro* data. Minocycline k_{drug} was 22% lower *in vivo* than *in vitro*, likely reflecting the reduced *in vivo* k_{growth}. The combined effect was well captured for the final model, and there was no significant difference between *in vitro* and *in vivo* in the drug interaction parameters. The fit of the final integrated PK/PD model to the three datasets is shown as VPC plots in Fig. 3 (*in vitro* part) and in Supplementary Fig. S6 (*in vivo* part). Fig. 4 displays the GOF of the *in vivo* data set, showing the predicted individual bacterial concentration–time curves. The model code of this final integrated PK/PD model is provided in Supplementary Text S3.

3.4. Prediction of drug effect in humans with ARU705 PK/PD model

The predictions using PK models for polymyxin B [16] and minocycline from either Welling et al. [17] or Lodise et al. [14] give similar results, and the latter are shown in Fig. 5. It can be seen that predictions based on model parameters estimated from static *in vitro* data with or without dynamic *in vitro* data were similar (upper two panels), and also similar to that from the *in vitro* part of the combined *in vitro*/*in vivo* data (lower left panel). They all indicated a benefit of the combination compared with monotherapy, achieving approximately 5 $\log_{10}$ CFU/mL reduction over 24 h. In comparison, predictions based on the model parameter estimates from *in vivo* data only resulted in approximately 2 $\log_{10}$ CFU/mL reduction from the start of treatment under combination therapy (lower right panel). Advantages of the combination treatment over the monotherapies were evident, as in all cases, the latter reached Bmax at 24 h. As expected from *in vivo* vs. *in vitro* parameter estimates, the predicted effect of polymyxin B monotherapy based on the *in vivo* part was lower than that based on the *in vitro* part of the model, with less pronounced initial killing in the first hour followed by extensive regrowth. The predicted higher effect of minocycline monotherapy based on the *in vivo* part compared with that based on the *in vitro* part of the model would be a reflection of the lower *in vivo* bacterial growth rate, as the killing effect *in vivo* was slightly lower than that *in vitro* (Table 3).

4. Discussion

This study illustrated a model-informed translational approach, using semi-mechanistic PK/PD modelling, to evaluate the antibiotic combination effect of polymyxin B and minocycline against two carbapenamase-producing *K. pneumoniae* strains with different susceptibilities. This article has shown how the information collected from *in vitro* and *in vivo* experimental models can be integrated to help predict bacterial killing in humans. The bacterial growth and antibacterial effect (both in monotherapy and in combination) across experimental systems were quantified, and the translational capacity of the PK/PD models across the experimental systems was demonstrated.

The previously developed PK/PD model structure was applied successfully to ARU705, which has distinct MIC values and resistance mechanisms (OXA vs. KPC producing) compared with the strain from which the model was built (ARU613) [8]. For both strains, the model could well predict dynamic time-kill experiments based on model parameter estimates from static experiments alone (a preliminary exploration of such translational capacity was reported previously [9]). However, the translation from *in vitro* to *in vivo* settings had to be adjusted by allowing some parameter estimates to be separated for the two systems, which reflected the discrepancy between them. Such discrepancy resulted in a difference in the predicted drug effect in humans. Nevertheless, all predictions indicated a beneficial effect of the combination in humans compared with monotherapy.

Population analysis profiling from *in vitro* dynamic time-kill experiments and whole genome sequencing of the strains [9] revealed that both pre-existing resistant subpopulations (with different resistance levels) in the inoculum and reversible or irreversible acquired resistance after exposure could be the cause of regrowth. A resistance model structure characterized by k_{on} (and k_{off}) for resistance development (and reversion) [8], and reflecting the increase of antibacterial resistance over time, best described the data and may summarize different types of resistance.

A similar translational PK/PD modelling approach across *in vitro* and *in vivo* experimental designs has been suggested previously [5,6,18–20] for monotherapies, and is shown in this study for drugs used in combination. This reinforces the value of semi-mechanistic PK/PD models in translation to integrate the knowledge and data gained from preclinical settings. The conventional PK/PD index approach for translation, referred to in regulatory guidance [21], relies on the drug effect (PD) targets (PDTs) derived from mouse infection models to predict doses in humans. The authors believe that a PK/PD modelling approach for translation, as illustrated in this study, is a step forward. This is because PDTs (a single time point value, usually at 24 h) do not describe the impact of bacterial growth and killing over time. This may be a particular limitation for combination studies where the relative drug contribution to the bacterial killing changes over time [22], and differences in murine and human PK may have an even greater impact on the interaction. PK/PD-model-based translation may therefore enable more precise prediction of clinical effects.

Table 3
Parameter estimates for pharmacokinetic–pharmacodynamic models from different steps, reported as typical values (RSE).

Parameters	Description	*in vitro* ARU613		*in vitro* ARU705		*in vitro* and *in vivo* ARU705	
		Static[a]	Static + dynamic (M1)	Static (M2)	Static + dynamic (M3)	Static + dynamic + mice (M4)	
						In vitro	*In vivo*
Bacteria-related parameters							
k_{growth} (/h)	Rate constant of bacterial growth	1.37 (13%)	1.32 (14%)	1.15 (20%)	0.971 (14%)	0.971 FIX[d]	0.690 FIX[d]
k_{death} (/h)	Rate constant of natural bacterial death	0.179 FIX	0.179 FIX	0.179 FIX	0.179 FIX	0.179 FIX	0.179 FIX
B_{max} (CFU/mL)	Maximum bacterial concentration in system	$10^{9.53}$ (1%)	$10^{9.42}$ (1%)	$10^{9.41}$ (1%)	$10^{9.58}$ (2%)	$10^{9.58}$ FIX[d]	$10^{10.3}$ FIX[d]
T_{lag} (h)	Lag time for bacteria to transfer from S to R	0.304 (26%)	0.296 (28%)	0 FIX	0 FIX	0 FIX	0 FIX
Minocycline-related parameters							
$Slope_1$ (L/mg/h)	Slope in power function for k_{drug}	0.339 (27%)	0.312 (29%)	0.285 (40%)	0.378 (27%)	0.448 (5%)	0.349 (8%)
γ_1 (-)	Exponent in power function for k_{drug}	0.546 (10%)	0.568 (10%)	0.921 (15%)	0.850 (16%)	0.698 (7%)	Shared
$Slope_2$ (L/mg/h)[b]	Slope in power function for k_{on}	0.179 (33%)	0.174 (39%)	0.00134 (8%)	0.0437 (29%)	0.221 (22%)	Shared
r	Exponent in power function for k_{on}	1 FIX	1 FIX	5 FIX	3.67 (5%)	2.90 (5%)	Shared
$Slope_3$ (-)	Slope in linear function for adaptive resistance	-10.2 (43%)	-11.5 (49%)	-2.29 (26%)	-3.13 (39%)	-2.12 (20%)	Shared
Polymyxin-B-related parameters							
$Slope_1$ (L/mg/h)	Slope in power function for k_{drug}[c]	0.0690 (31%)	0.0690 (33%)	8.50 (25%)	11.5 (39%)	12.5 (20%)	3.66 (19%)[c]
γ_1 (-)	Exponent in power function for k_{drug}[c]	1.20 (6%)	1.20 (6%)	1.30 (5%)	1.38 (3%)	1.38 (2%)	20 FIX[c]
EC_{50} (mg/L)	EC_{50} for k_{drug}	NA	NA	NA	NA	NA	3.71 (4%)[c]
$Slope_2$ (L/mg/h)	Slope in linear function for k_{on}	0.00402 (16%)	0.00423 (17%)	0.590 (53%)	1.23 (56%)	1.33 (28%)	0.0324 (22%)
$Slope_3$ (-)	Slope in linear function for adaptive resistance	1 FIX	1 FIX	1 FIX	1 FIX	1 FIX	1 FIX
Combination-related interaction (polymyxin B affecting minocycline)							
E_{max} (-)	E_{max} for $k_{drug,MIN,COMB}$	2.45 (28%)	2.47 (32%)	5.39 (82%)	2.86 (40%)	1.76 (19%)	Shared
EC_{50} (mg/L)	EC_{50} for $k_{drug,MIN,COMB}$	0.285 (71%)	0.271 (94%)	0.316 (137%)	0.273 (37%)	0.229 (28%)	Shared
$Slope_4$ (-)	Slope for $k_{on,MIN,COMB}$	8.32 (27%)	7.81 (28%)	61.2 (75%)	68.9 (46%)	40.3 (33%)	Shared
γ_4 (-)	Exponent for $k_{on,MIN,COMB}$	0.479 (43%)	0.480 (54%)	0 FIX	1 FIX	1 FIX	1 FIX
Residual error (SD, log_{10} CFU/mL)		0.907 (7%)	0.895 (6%)	0.929 (11%)	1.06 (18%)	1.05 (7%)	Shared
Replicate residual error (SD, log_{10} CFU/mL)		0.164 (7%)	0.174 (6%)	0.220 (8%)	0.214 (12%)	0.214 (6%)	Shared

NA, not applicable; RSE, relative standard error (calculated by NONMEM sandwish matrix); Share: shared paramter with the *in vitro* part of the model.

[a] Parameters published in Zhao et al. Int J Antimicrob Agents 2020;55:105941.

[b] All parameters should be scaled with × 0.001.

[c] For *in vivo* part, k_{drug} was modelled by sigmoid Emax function: $3.66 \times C_{pmb}^{20}/(3.71^{20} + C_{pmb}^{20})$, where C_{pmb} is the polymyxin B concentration, 3.66 is the estimated Emax (/h), 3.71 is the estimated Ec50 (mg/L), and 20 is the fixed sigmoid factor.

[d] For *in vitro* part, the values were fixed to those from the model based on combined *in vitro* static and dynamic data. For *in vivo* part, the values were fixed to those estimated from *in vivo* growth control data.

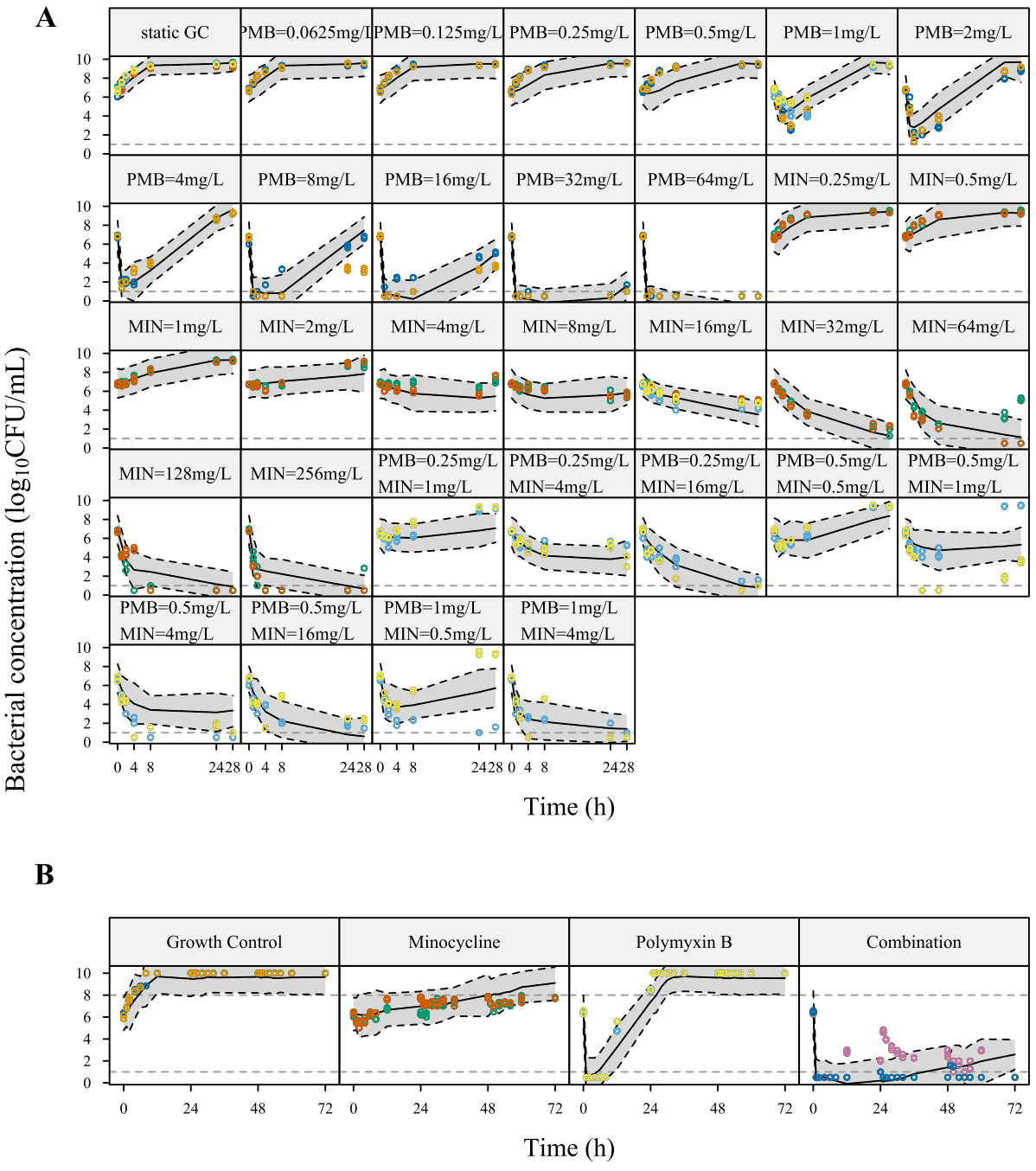

Fig. 3. Visual predictive checks for the *in vitro* (A) static and (B) dynamic time-kill experiment parts of the final pharmacokinetic–pharmacodynamic model (based on both *in vitro* and *in vivo* data) for ARU705, stratified by dose group. The observed bacterial concentrations (open circles, coloured by replicates), as well as the median (solid line) and its 95% confidence interval (grey shading) of the simulated data (n=500) are shown. The horizontal dashed lines are the limits of detection [for A and B, 1 $\log_{10}$ colony-forming units (CFU)/mL] or quantification (for B, 8 $\log_{10}$ CFU/mL). GC, growth control; PMB, polymyxin B; MIN, minocycline; COMB, combination.

With the quantified *in vitro*/*in vivo* discrepancy, as illustrated here, there would be a problem in translating the *in vitro* results directly to clinics. However, in light of the sparse nature of the *in vivo* data, and given that *in vitro* data generation is less resource demanding and allows for extensive repeated measurements and does not have the same ethical constraints as *in vivo* studies, the integration of *in vitro* data in PK/PD models can be much more valuable for preclinical to clinical translation. Through model-based translation, the links to be made between *in vitro* and *in vivo* data can be quantified and lead to more robust translation to clinics.

The discrepancy between the *in vitro* and *in vivo* settings, according to what was quantified by the PK/PD model, could be summarized as the difference in bacterial growth rate and the effect of antibiotic monotherapy. It is worth highlighting that no differences in interaction factors were found. The generalization of this finding that *in vivo* combination experiments could be reduced once an interaction term is quantified from *in vitro* data requires further research. Slower bacterial growth rate *in vivo* than *in vitro* (0.69 vs. 0.97/h) has been reported previously [5,6,23]. The discrepancies of the *in vitro* vs. *in vivo* antibiotic effects were not unexpected. In a previous study on the combination effect of polymyxin B and minocycline against *Acinetobacter baumannii*, not all strains showing a promising *in vitro* combination effect demonstrated the same effect *in vivo* [24].

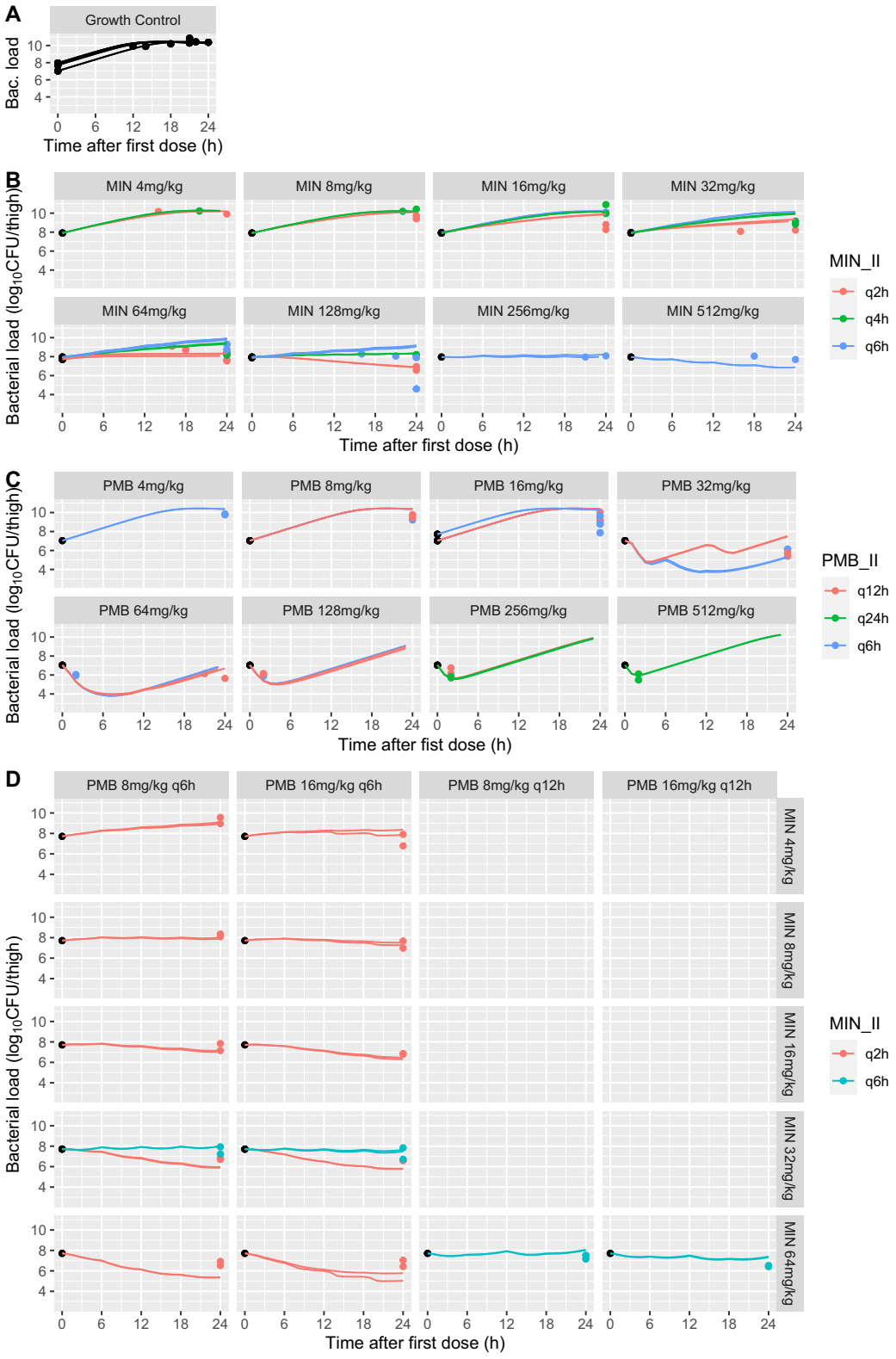

Fig. 4. Goodness-of-fit plot for the *in vivo* data and predictions from the final pharmacokinetic–pharmacodynamic (PK/PD) model (based on both *in vitro* and *in vivo* data) for ARU705, divided into control data (A), minocycline (MIN) monotherapy data (B), polymyxin B (PMB) monotherapy data (C), and combination data (D). The observed bacterial concentrations (solid circles), as well as the individual predictions (solid lines, predicted every 1 h) are shown, together with the start of treatment (black dots from respective batch). qnh, every n hours; II, dose interval.

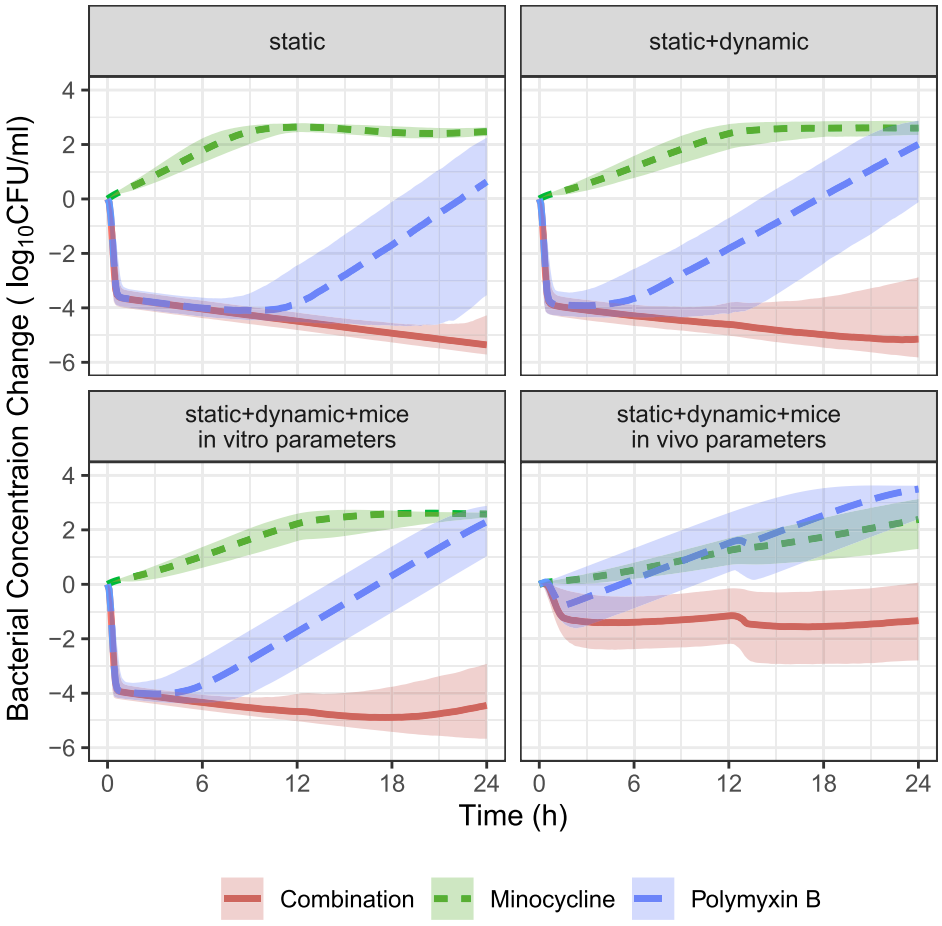

Fig. 5. Simulated bacterial concentration profiles (change from baseline) over time in patient plasma under the exposure of minocycline and polymyxin B as monotherapy and in combination using the parameter estimates from pharmacokinetic–pharmacodynamic models built from static time-kill experiments (top left panel), static and dynamic time-kill experiments (top right panel), and static and dynamic time-kill plus murine thigh infection model experiments (bottom panels). For the latter, simulations were conducted using *in vitro* and *in vivo* parameters, respectively. The median (lines) and the 80% prediction interval (shaded area) of the simulated data (n=1000, every 0.1 h) are shown. CFU, colony-forming units.

This could be due to a low penetration ratio to the target site *in vivo*.

The consistency between *in vitro* static and dynamic parameter estimates demonstrated the feasibility of the PK/PD model built from static data in translating to dynamic data. However, collecting data from dynamic experiments where PK profiles are mimicked could complete the preclinical package. *in vitro* dynamic experiments that can assess the effects of multiple different PK profiles have been recommended by the European Medicines Agency as part of the non-clinical PK/PD studies [21]. *in vitro* dynamic experiments, which could be viewed as a step closer to *in vivo* experiments compared with static experiments, could help identify potential drug effects that may be difficult to observe with static drug concentrations alone. Such experiments could also help understand whether the differences between *in vitro* and *in vivo* conditions are due to the dynamics of concentration or other factors inherent to each system.

The present polymyxin B PK model had a two-compartment structure, as reported previously [25], but the absorption model differed. Here, an increase in the dose of polymyxin B resulted in an exponential decrease in the first-order absorption rate constant. The tested doses (0.5–64 mg/kg) had a wider range than in the dataset used to develop the published model (2–32 mg/kg), where a parallel first-order and saturable absorption model was used to describe the data [25]. The elimination could also be simplified to one saturable pathway, rather than parallel linear and saturable elimination [25]. Another study with doses ≤12 mg/kg reported a one-compartment model with linear absorption and elimination [26]. Despite the differences in model structure, the reported values of volume of distribution were similar, at approximately 25 mL (for a 22-g mouse). Clearance was slightly higher in the present study; approximately 6–13 mL/h compared with 4–7 mL/h [25,26] for concentrations ranging between 1 and 10 mg/L (for a 22-g mouse). The different polymyxin B components (B1, B1-Ile, B2 and B3) were not separated in the present study, but it has been reported previously that they have similar PK profiles in murine plasma [26]. The free plasma concentrations in mice in the *in vivo* experiments predicted based on the developed PK models (Supplementary Fig. S1) were comparable to the concentrations used in the static experiments (0.0625–64 mg/L), and covered the concentrations used in the dynamic experiments which mimicked free plasma concentrations in humans (Table 1). A dose of 32 mg/kg q12h in mice led to trough and peak concentrations of approximately 0.5 and 5 mg/L, respectively, and was close to the concentration range in humans.

For minocycline, the inclusion of relative bioavailability (F) improved the PK model fit significantly (dOFV=70). F decreased with an increase in the dose of minocycline, which may be due to solubility issues (see Section 3.1). Non-linear clearance was not apparent in the present study, including doses up to 64 mg/kg, while

saturable clearance has been reported for doses of 100 mg/kg [27]. The predicted free concentrations in mice (mostly <2 mg/L, Supplementary Fig. S1) were slightly lower than those from *in vitro* dynamic experiments [trough (C_{min}) approximately 1.8 mg/L, Table 1], where PK profiles from the model by Welling et al. [17] was targeted. A dose of 64 mg/kg q6h in mice led to trough and peak concentrations of approximately 0.5 and 1 mg/L, respectively, which is close to the concentration range in humans predicted by the population PK model developed by Lodise et al. [14].

One limitation of this study was that translation was only explored in two strains, and only one of the strains was sufficiently susceptible to study *in vivo*. However, considering the wide range of experiments that would be needed, the addition of another strain would be challenging given the available resources and ethics. Although this may limit the generalizability of the concluded benefit of the combination of minocycline and polymyxin B, this study is sufficient to demonstrate the translational PK/PD analysis approach for antibiotic drug combinations, and showcase how to maximize the utility of the data collected from different stages of the experiments in a continuous and interactive learn-and-confirm process. Another limitation is that the present explanation of the discrepancy between *in vitro* and *in vivo* systems was primarily data driven. More research to provide a better mechanistic understanding of the differences between *in vitro* and *in vivo* data and human systems is warranted. Since obtaining a deeper mechanistic understanding is usually challenging, the robustness of such data-driven results could be enhanced by taking more repeated samples over time during *in vivo* experiments instead of focusing solely on the 24-h time point. This would facilitate better translation and prediction of bacterial killing in patients.

5. Conclusion

This study illustrated the capacity of a developed PK/PD model in facilitating translation of the combined effect of polymyxin B and minocycline against *K. pneumoniae* across various experimental settings. The combination was predicted to have a better antibacterial effect in humans than either monotherapy using model parameter estimates from both *in vitro* and *in vivo* data. The results suggest the need for further investigations into the potential clinical value of this combination as a last-resort regimen against carbapenemase-producing *K. pneumoniae*.

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Ethical approval: Approval by the institutional animal welfare body, Erasmus Laboratory Animal Science Centre, Rotterdam (Licence No. AVD101002016702), in accordance with the EU Animal Directive (2010/63/EU 2010).

Author contributions: Conceptualization: CZ, EN, LF. *in vitro* experiments: AO, CM under the supervision of PL and TT. *in vivo* experiments: SB, AM. Drug assay: VAC. PK and PK/PD modelling and simulation: CZ, ZW under the supervision of EN, LF. Writing – original draft: CZ and LF. Writing – review and editing: all authors.

Declaration of competing interests: None declared.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2025.107443.

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