



Introduction

Background

Pharmacokinetic-Pharmacodynamic (PKPD) models built on preclinical data can be a useful tool for efficient exploration of antibiotic effects [1]. Polymyxin B (PMB) is an antibiotic mainly used for the treatment of infections caused by resistant gram-negative bacteria, and is regarded as an antibiotic of last resort.

Objective

The aim of this work was to explore if an *in silico* semi-mechanistic PKPD model for PMB and *Klebsiella Pneumoniae* (*K.p*) built on *in vitro* data from static concentrations [2], can be extrapolated to predict *in vivo* data in mice by incorporating an *in vivo* PK model.

Materials and Methods

PK Studies

- Plasma concentrations were sampled 0-8 h post-dosing from a thigh infected mice model with PMB (0.5-64 mg/kg, 2 mice per sampling point) administered subcutaneously.
- Data below the limit of quantification (LOQ=0.2 mg/L) were included in the modeling (M3 method).
- A PK model was developed and applied to be linked with PMB PD model. The fraction of unbound-drug in plasma was fixed to 8.6% [3].

PKPD Studies

- Five *K.p* isolates (MIC 0.5-2 mg/L) in mice were exposed to PMB 1-32 mg/kg q6h. In addition, for *K.p104* 8-64 mg/kg q12h was studied. Thighs were homogenized and plated 24 h post-dosing, colony forming units (CFU) were counted after over-night incubation. Growth controls in untreated mice were also included.

PKPD model for *in vivo* data

- An *in vitro* PD model developed from a resistant *K.p* strain (MIC 16 mg/L) [2] linked with the developed *in vivo* PK model was applied to predict *in vivo* observations.
- Bacterial growth rate constant (k_{growth}) and maximal bacterial amount (B_{max}) were re-estimated based on CFUs observed in growth controls to adjust to *in vivo* conditions.
- 1st step: The Slope, describing the PMB killing effect, was scaled by MIC difference between the *in vitro* and *in vivo* strains. 2nd step: The Slope and γ describing the PMB killing effect, were allowed to be re-estimated to explain differences between the *in vivo* and *in vitro* systems. CFU counts predicted by the PKPD model were compared to the observed.

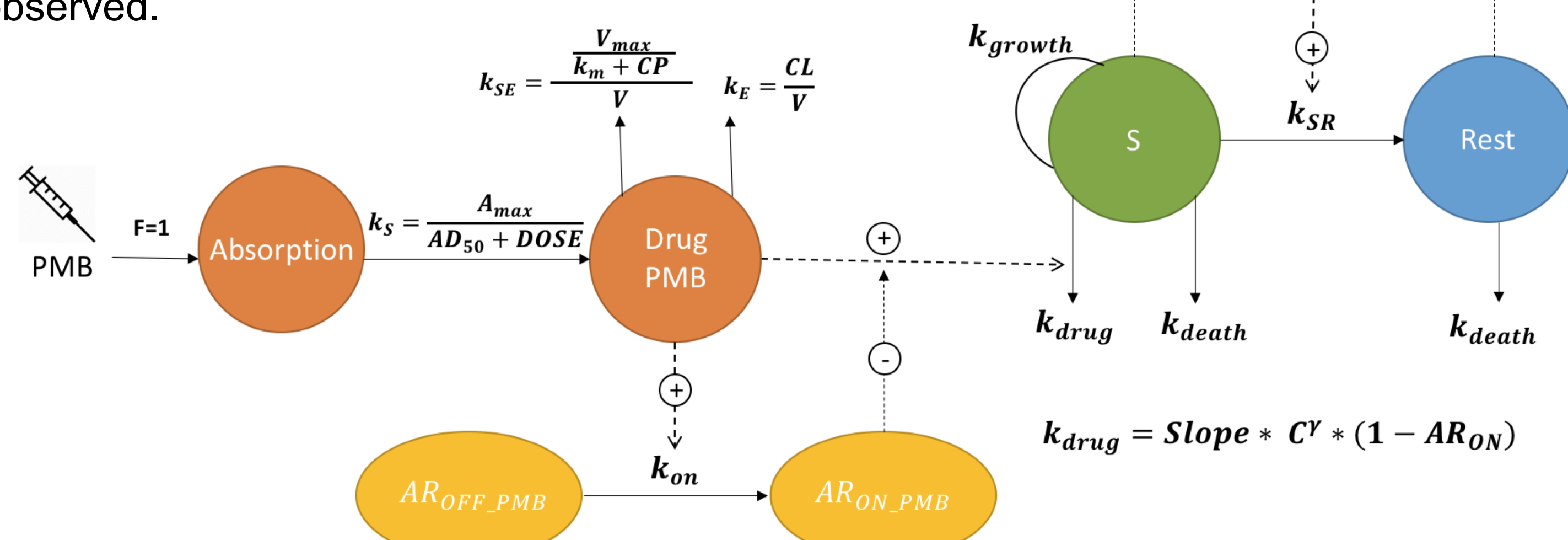


Fig 1. Schematic illustration of the semi-mechanistic PKPD model for PMB against *K.p* *in vivo*. The PK profile of PMB in mice was well described by a PK model comprising of a saturable absorption pathway and parallel linear and capacity-limited elimination. Bacteria transferred between drug-susceptible, growing bacterial compartment (S) and non-drug-susceptible, non-growing bacteria compartment (Rest). Drug killing effects (Drug) are decreased by adaptive resistance (AR). The rate for development of AR (k_{on}) was stimulated by drug concentration.

Results

PK modeling

- Overall 139 PMB concentrations were available, and well described by a PK model comprising of a saturable absorption pathway with the maximum absorption rate constant (A_{max}) decreasing with higher doses, and parallel linear and capacity-limited elimination. (Fig 1 & Fig 2 & Table 1)

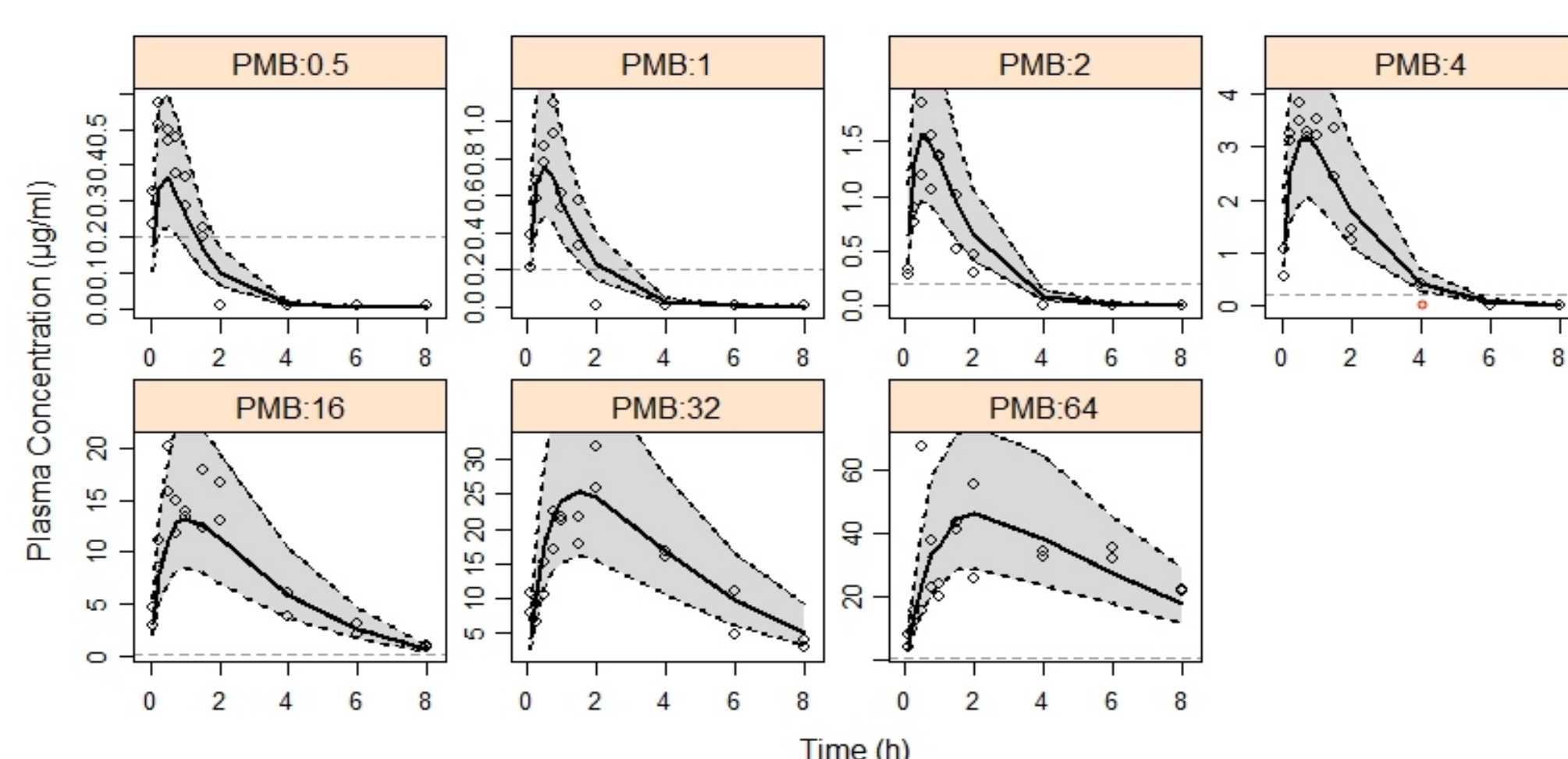


Fig 2. Visual predictive checks (VPC) grouped by PMB dose 0.5-64 mg/kg for the final PK model. The observed plasma concentration (open circles), the median (solid line) of the simulated data, as well as 95% confidence interval (grey shade) of the median are shown. Limit of quantification (LOQ) is also plotted (horizontal dashed line).

PKPD model for translation *in vitro* - *in vivo*

- In total, 84 *in vivo* CFU counts were available, including 20 CFU counts from growth control experiments and 64 CFU counts following various PMB exposures.
- The estimated *in vivo* k_{growth} was 51% lower than the earlier estimated *in vitro* value (0.676 vs. 1.37 h⁻¹). B_{max} was estimated to 9.34 log₁₀ CFU/thigh.
- Fig 3 shows the model fit when the PMB killing effect (k_{drug}) is scaled by MIC, an *in vitro* measurement of strain susceptibility. The sensitivity of predicted log₁₀CFU when MIC varies from 0.5 to 2 fold of mean measured MIC *in vivo* was explored.
- Model predictions were significantly improved (dOFV=-112.7, df=2) after re-estimation of both slope and γ of PMB killing effect (k_{drug}) compared to scaling by MIC. (Fig 4)

Table 1. Model parameters (RSE, Relative Standard Error)

PK Parameter	Description	Estimation (RSE)
A_{max} (mg/h)	Maximum rate of saturable absorption	1.85 (21%)
AD_{50} (mg)	Amount in absorption compartment at $\frac{1}{2} A_{\text{max}}$	0.376 (29%)
V (L)	Apparent central volume of distribution	0.0230 (4.9%)
CL (L/h)	Apparent linear elimination clearance	0.00363 (13%)
V_{max} (mg/h)	Maximum rate of saturable elimination	0.0349 (20%)
K_m (mg/L)	Plasma concentration at $\frac{1}{2} V_{\text{max}}$	1.62 (29%)
Additive residual error on log-transformed data (SD, log mg/L)		0.350 (7.0%)
PKPD Parameter	No Estimation (RSE)	Estimation (RSE)
k_{growth} (h ⁻¹)	Rate constant of bacterial growth	0.676 (3.9%)
k_{death} (h ⁻¹)	Rate constant of natural bacterial death	0.179 (fixed)
B_{max} (CFU/thigh)	Maximum bacterial amount in system	$10^{9.34}$ (1.2%)
Slope 1 (L/mg/h)	Slope in power function for k_{drug} of <i>K.pWT</i>	2.21 ^a
Slope 2 (L/mg/h)	Slope in power function for k_{drug} of <i>K.p17</i>	1.10 ^a
Slope 3 (L/mg/h)	Slope in power function for k_{drug} of <i>K.p58</i>	0.552 ^a
Slope 4 (L/mg/h)	Slope in power function for k_{drug} of <i>K.p104</i>	0.552 ^a
Slope 5 (L/mg/h)	Slope in power function for k_{drug} of <i>K.p74</i>	0.552 ^a
γ	Hill factor in power function for k_{drug}	1.20 ^b
Additive residual error on log-transformed data (SD, log ₁₀ CFU/thigh)		1.47 (4.4%)
		1.00 (4.6%)

^a Equation of scaling : $\text{In vivo Slope} = \frac{\text{In vitro MIC}}{\text{In vivo MIC}} * \text{In vitro Slope}$
^b Fixed to value reported in the *in vitro* model

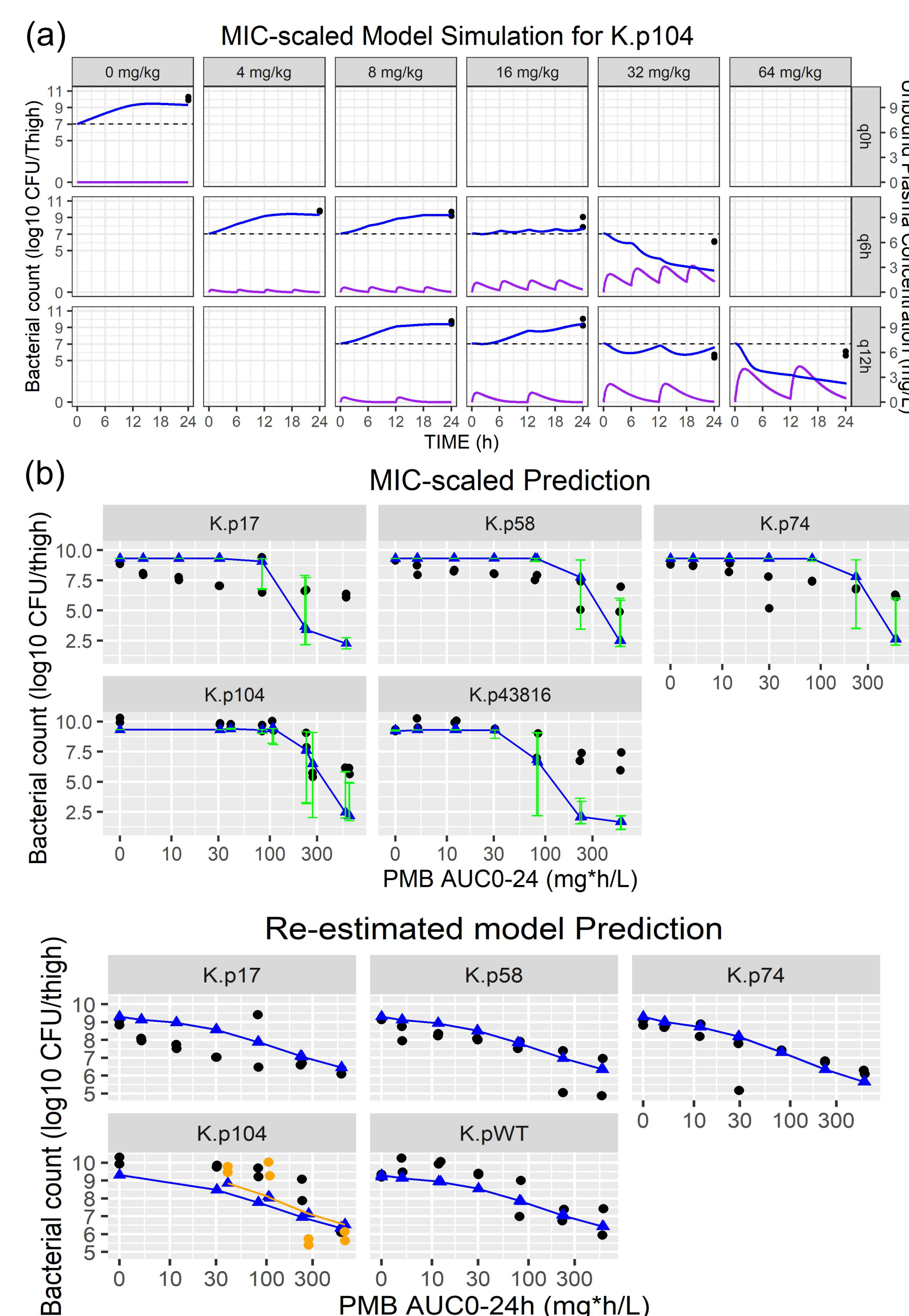


Fig 3. PKPD model predictions *in vivo*. Slope was scaled by difference in MIC between the strains.

(a) PKPD model prediction for *K.p104*. Predicted plasma unbound-drug concentration (—), predicted bacterial count (—) (log₁₀ CFU/thigh) over time, and observed bacterial count (log₁₀ CFU/thigh) at time 0 h (—) and 24 h (●) are shown.

(b) Predicted (▲, —) and observed (●) bacterial count vs. total drug exposure (AUC₀₋₂₄) are shown. The range of predicted bacterial count, when MIC varies from 0.5 to 2 fold the mean of measured MIC *in vivo* is illustrated (■).

Fig 4. PKPD model predictions *in vivo* when slope and γ in power function of k_{drug} were re-estimated. Predicted (▲, —; from q6h, — from q12h) and observed (● from q6h, ● from q12h) bacterial count vs. total drug exposure (AUC₀₋₂₄) are shown.

Conclusions

- The PK of PMB in mice was well described by a PK model comprising of a saturable absorption pathway and parallel linear and capacity-limited elimination.
- The PKPD model built on *in vitro* data could predict the effect data of PMB against *in vivo* *K.p* infectious model data reasonably well after re-estimation of k_{growth} , B_{max} . (Fig 3)
- The model fit improved further when slope and γ were allowed to be re-estimated. The re-estimated low γ implies that the concentration-effect relationship appears to be shallower *in vivo* and the differences in susceptibility between the strains observed *in vitro* are less pronounced *in vivo*.
- The predictive performance of the *in vitro* PKPD model will be further tested in combination with rifampicin and minocycline in *in vivo* models infected by *K.p104*.

References

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