Pharmacokinetic - Pharmacodynamic Modeling of Pre-existing and Emerging Resistance of *Pseudomonas aeruginosa* to Colistin

UPPSALA UNIVERSITET

Ami Mohamed (1), Otto Cars (2) and Lena E. Friberg (1)

(1) Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden
(2) Department of Medical Sciences, Uppsala University, Uppsala, Sweden

Background and Objective

In recent years, colistin has gained popularity as a last resort antibiotic in the battle against resistant bacteria. *Pseudomonas aeruginosa* is well known to develop resistance against multiple antibiotics and thus, there is a need to ensure proper dosing of colistin either as monotherapy or in combination with other antibiotics. As colistin is administered as CMS, a prodrug, there is a delay before efficient concentrations are obtained and a loading dose may be warranted.

The aim of this study was to develop a pharmacokinetic-pharmacodynamic (PKPD) model that describes the time course of the bactericidal activity of colistin against wild-type and resistant *P.aeruginosa in vitro*, and to investigate the bacterial kill after different dosing schedules based on PK in patients and the developed model.

Methodology

Time –kill curve experiments:

- In-vitro time kill curve experiments were conducted for 24 hours on two strains of *Pseudomonas aeruginosa*, wild-type (ATCC 27853), MIC of 1 mg/L, and a clinically isolated resistant-type (PL0603761) with MIC of 1.5mg/L.
- Colistin exposure was at different initial concentrations ranging between 0.25-16 times the MIC. Actual colistin concentrations were measured at 0, 8 and 24 hours by LCMS-MS (1)
- Bacterial counts were monitored with frequent sampling and conducted in two to three replicates.

Data Analysis & Model Building:

- All log-transformed data were fitted simultaneously using NONMEM7 with LAPLACIAN and M3 method for handling data below level of detection.
- The semi-mechanistic model includes:
  - compartments for drug-susceptible, growing bacteria (S) and for insusceptible, resting bacteria (R) with a breakpoint for turning on the rate of transfer of bacteria (k_{SR}) between the two compartments (2,3),
  - different models for the apparent emergence of resistance were tested; a binding function that inhibits the power effect of colistin (3), a compensatory mutation function (4) or an estimated pre-existing fraction of resistant bacteria in the inoculum (5).
- Assumption of no variability between experiments but with quantified residual error accounting for replicates (L2) data item.

Predictions of dosing schedules:

Bacterial counts were predicted for a typical individual by allowing the concentrations predicted by a previously developed PK model for the prodrug, colistin methanesulfonate (CMS) and colistin (6) to drive the bacteria kill. Concentration-dependant protein binding was also accounted for based on an equation derived from an equilibrium dialysis study.

Results

Colistin binding:

- The measured colistin concentrations were 4.4–78% lower than the intended concentrations and there was a progressive reduction of the concentration with time due to unspecific binding of colistin to material and possible degradation during the experiments.

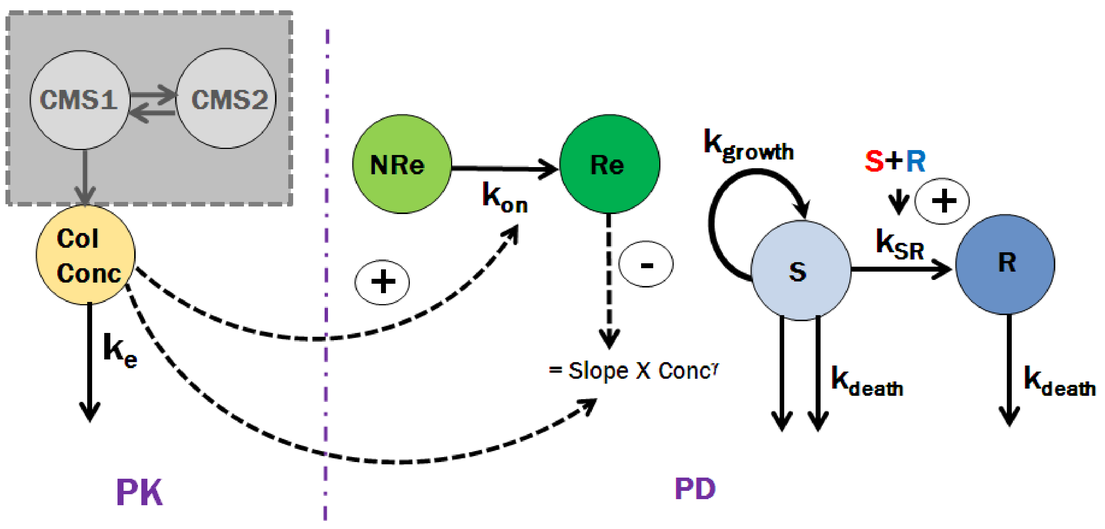

Fig. 1: PKPD model for colistin with developing resistance. The two CMS compartments (in grey box) were only utilised during predictions.

Model building:

- The developed model (Fig 2) could describe the data for both strains of *P. aeruginosa*. The application of actual colistin concentrations and the rate of loss in the modeling was important in the characterization of the concentration-effect relationship.
- The emergence from non-resistance (NRe) to resistance (Re) in the experiments was best described by a binding function (4). The drug effect was best described by a power function; for wild-type: 6.2 X Conc$^{0.66}$ and for the resistant strain: 1.0 X Conc$^{1.2}$. The growth rate, k_{growth}, was 31% lower in the resistant strain. The rate of resistance development, k_{on}, was a linear function dependent on concentration with an assumption of no resistance reversibility for both strains.

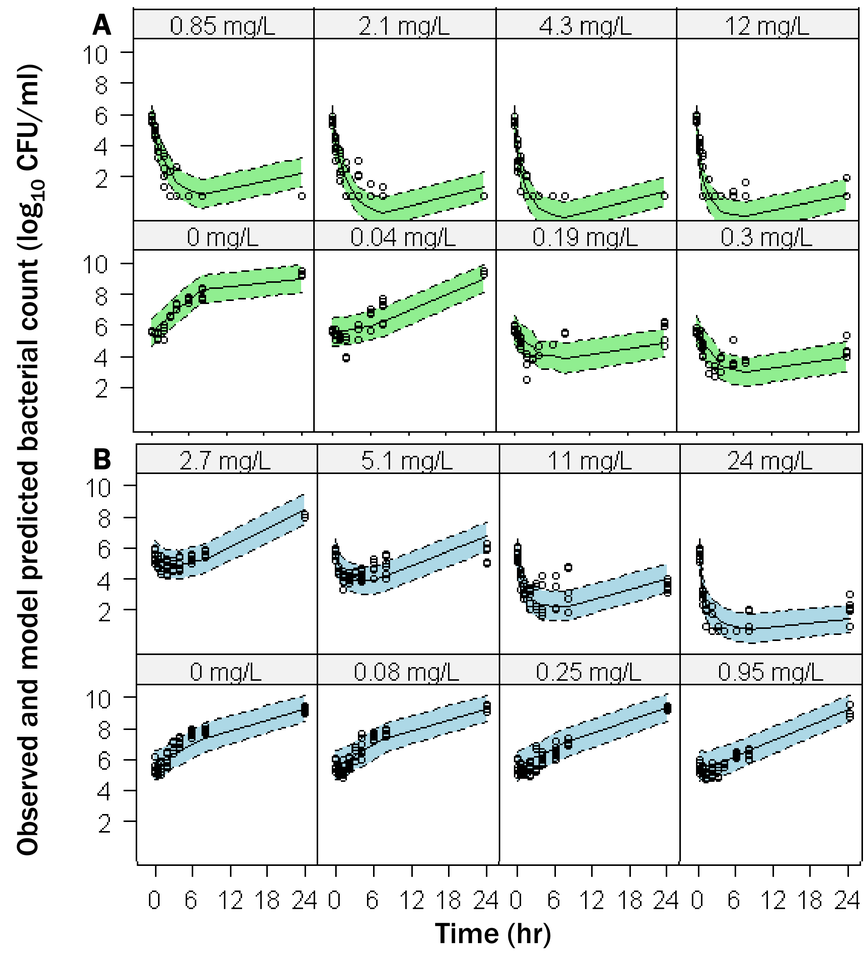

Fig. 2: Visual predictive checks for the different strains of *P.aeruginosa* in diffferent initial concentrations of colistin

A. Wild type

B. Resistant strain

Model evaluation:

- The objective function value was low for the binding function compared to the other evaluated models.
- VPCs showed the adequacy of the model for both wild-type and the resistant bacteria strain(Fig. 2).

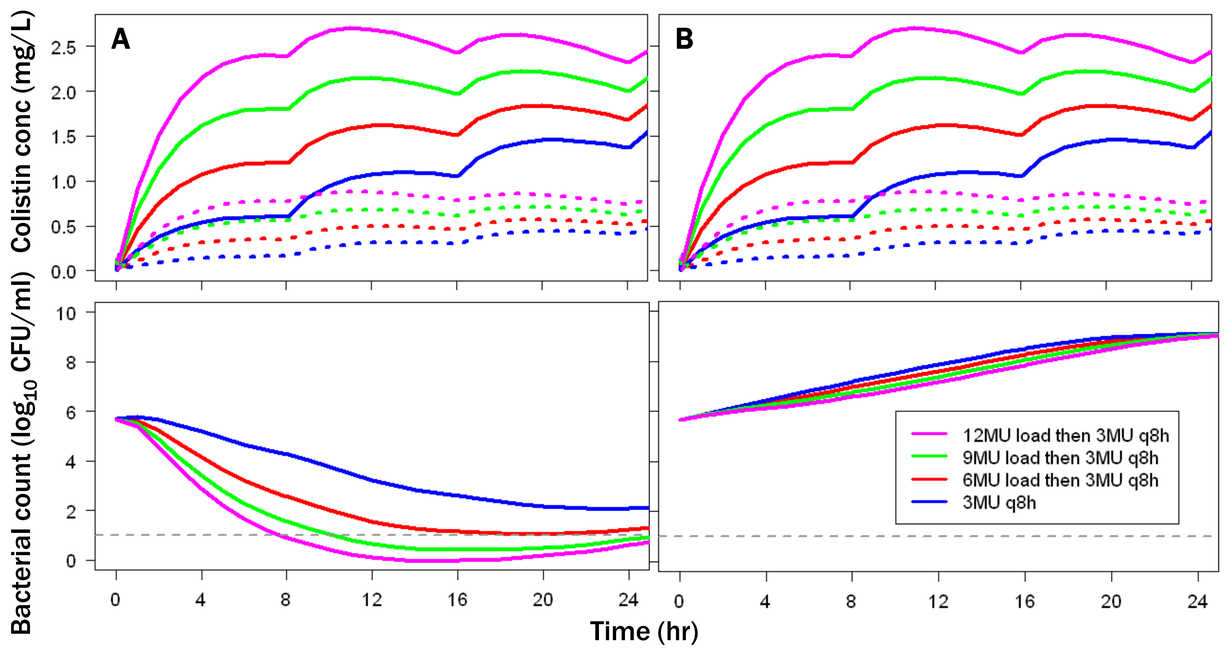

Fig. 3: Model predictions of colistin concentration (top panel) for a typical individual receiving CMS 3MU 8 hourly or an initial 6 MU, 9 MU or 12MU as loading dose followed by 3MU 8 hourly (all doses were given as 15 minutes infusion) with *P.aeruginosa* count (lower panel). A. Wild type B. Resistant strain. (— Total colistin concentration; ----- Unbound colistin concentration). Bacterial count below the limit of detection are plotted at 10 CFU/ml (grey dashed line).

Model predictions:

- The predicted unbound concentrations of colistin were 18 - 33% lower than the total concentrations at clinical relevant concentrations.
- For the wild-type bacteria, it was predicted that it took 10 hours to reach a bacterial count of $log_{10}2$ following a loading dose of 6MU CMS. For 3MU, the corresponding time was 22 hours.
- None of the dose levels was sufficient to reduce the resistant bacterial counts.

Conclusions

- The PKPD model for colistin described bacterial kill for both wild-type and resistant isolates,
- The model will be valuable in further exploration of potential dosing regimens for example longer infusion period or a higher maintenance dose (eg 4.5MU every 12 hourly).
- For the resistant bacteria, clinical exposure would not be sufficient and a combination with other antibiotics is indicated.

References

1. Jansson et al. *Journal of Pharmaceutical and Biomedical Analysis*. 2009; 49: 760–767.
2. Nielsen EI et al. Antimicrob Agents Chemother. 2007; 51:128-136.
3. Mohamed A et al. Pharmacokinetic-Pharmacodynamic Model for Gentamicin and its Adaptive Resistance with Predictions of Dosing Schedules in Newborn Infants (Manuscript)
4. Wu B et al. *Pharmazie*. 2010;65:417
5. Khan D et al. PKPD-Modeling of time-kill curves from E. coli mutants exposed to ciprofloxacin. PAGE Abstract 2011.
6. Plachouras et al. *Antimicrob Agents Chemother.* 2009; 53(8):3430-3436.

Contact: ami.mohamed@farmbio.uu.se