Comparing an adaptation with a mutation modelling approach to assess resistance development of antibacterials *in vitro*.

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Background

- *In vitro* static concentration time kill experiments (SCTK) experiments are commonly conducted during early preclinical development.
- Adaptation models [1] or mutation models [2] have been used to quantify concentration-effect relationships.
  - To our knowledge no comparison of the two methods has been made yet.
- Already early in the preclinical phase, resistance development of bacteria against antibacterials can be observed.
  - Use of PD models for rapid screening and/or mechanistic insight in the resistance development is important.

Objective

- Evaluate the utility of two PD modelling approaches to describe resistance development of antibacterials based on SCTK data and consequently
  - describe the time course of bacterial count
  - perform preliminary prediction of human dose for early stage decision making.

Methods

- The bacterial count-time profiles were analyzed with NONMEM
  - BLOQ data (<10 CFU/ml) was handled with the M3 method.
- Final models were compared using AIC.
- Simulations were performed using Berkeley Madonna to predict human dose:
  - Assumption: all PD parameters are scaled directly to humans
  - Based on known human PK (unbound concentration) doses were determined to obtain
    - -1 or -2 log kill after 24h (end of SCTK experiment)
    - stasis after 5 days (typical duration of administration in patients)

Compounds & bacterial strains

- Novel siderophore conjugated Beta-Lactams: MB1, MB2
- Novel LpxC inhibitor: LpxC1
- Beta-Lactam: Meropenem (on the market since 1996)

MIC in µg/ml		MB1	MB2	LpxC1	Meropenem [3]
Klebsiella pneumoniae	MIC_{90}	8	2	0.5	
KP-1487	MIC	4	8	2	
Pseudomonas aeroginosa	MIC_{90}	1			4
PA-UC12120	MIC	1			1

Data

In vitro static concentration time kill experiment:

- Baseline bacterial count: 10^5 CFU/ml.
- Samples: 0, 2, 4, 6, 8, 10, 12, 24, and 26 h.
- Conc: 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 µg/ml.
- n=1 per conc.

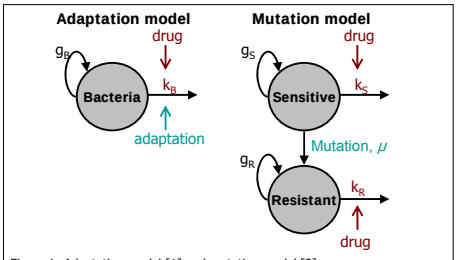

Figure 1, Adaptation model [1] and mutation model [2].

Model

- Bacterial growth was described by Simplified Richards growth or Gompertz growth [4].
- Adaptation model:
  - Emax or Sigmoidal Emax concentration-effect relationship:

$$k_B = k_{max} \frac{C^\gamma}{C^\gamma + (EC_{50}\alpha)^\gamma}$$

  - Resistance development was described by changes of EC_{50} with time and/or compound concentration.
  - The full adaptation function [1] and simplifications were considered:

$$\alpha = 1 + \beta(1 - \exp(-ZCt))$$

- Mutation model.
  - Different concentration-effect relationships (E_{max}, Sigmoidal E_{max}, linear, log-linear, no kill) were examined for the kill of susceptible, k_S and resistant k_R bacteria.
  - Initial count of resistant bacteria was assumed to be 0.
  - Mixture model with two populations was introduced to describe the (stochastic) mutation.
    - Mixture probability for at least one mutation before the end of the experiment [5]:

$$E[M] = \mu S_0 \frac{g_S}{g_S - k_S}(g_S - k_S)(\exp((g_S - k_S)T_{end}) - 1)$$

$$P(POP1) = 1 - \exp(1 - E[M])$$

    - The first resistant bacteria for POP1 is dosed into the resistance compartment with the lag-time [5]:

$$LAG = \frac{1}{g_S - k_S}\log((g_S - k_S)/(S_0 g_S \mu) + 1)$$

    - Hereafter the growth, kill and (deterministic) mutation for the resistant bacteria is described by an ordinary differential equation.

Results

- **The adaptation model with simplified adaptation functions and the mutation model both adequately captured the development of resistance during drug exposure (Figure 2 and 3, not all experiments shown).**
  - **3 out of 5 SCTK experiments were best described with the mutation model, based on the AIC (Table 1).**
- **For the mutation model, implementing mixture for the mutation was necessary to obtain realistic mutation rates estimated with good accuracy (Table 2).**
  - **The mixture probability decreases with increasing dose due to kill of the sensitive bacteria (Figure 3).**
- **No re-growth was observed at concentrations >4mg for all experiments.**
  - **MB1 vs PA-UC12120: No kill of the resistant bacteria → no mutations at high doses → no re-growth (Figure 3, right)**
  - **Other SCTK experiments: Kill of the resistant bacteria → no re-growth (Figure 3, left).**
- **Both approaches resulted in similar human dose predictions to obtain -1 and -2 log kill after 24h (1-4 fold difference) but were different in prediction of stasis after 5 days (2-6 fold difference) (Table 3 and Figure 4).**
  - **The clinical dose of Meropenem is 500-1000 mg which is close to the dose predicted by the adaptation model at stasis (day 5) or by the mutation model at -2 log kill (24h).**

Model comparison

Table 1, AIC for the adaptation and mutation models, respectively.

Compound	Bacteria	Adaptation	Mutation
MB1	PA-UC12120	89.28	59.60
MB1	KP-1487	44.59	52.07
MB2	KP-1487	17.40	22.96
LpxC1	KP-1487	35.42	6.44
Meropenem	PA-UC12120	37.23	12.41

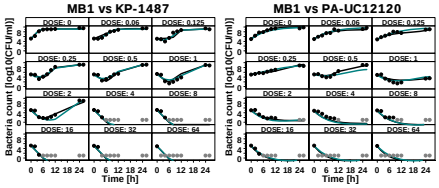

Figure 2, Prediction of the Klebsiella and Pseudomonas bacteria count with the adaptation model (turquoise) and mutation model (black). BLOQ observations are plotted at the limit of quantification (grey).

Mutation model

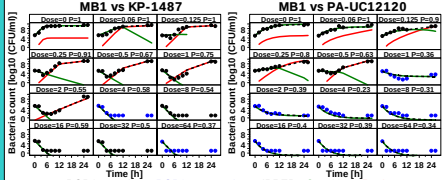

Figure 3, Prediction of the Klebsiella and Pseudomonas bacteria count with the mutation model. P is the mixture probability of at least one mutation for each dose (POP1).

Table 2, Estimated mutation rates (per cell division)

Compound	Bacteria	Mutation rate (CI)
MB1	PA-UC12120	$2.3 \cdot 10^{-6}$ ($6.8 \cdot 10^{-7}$-$7.8 \cdot 10^{-6}$)
MB1	KP-1487	$5.8 \cdot 10^{-7}$ ($4.7 \cdot 10^{-7}$-$7.1 \cdot 10^{-7}$)
MB2	KP-1487	$1.7 \cdot 10^{-6}$ ($8.5 \cdot 10^{-7}$-$3.2 \cdot 10^{-6}$)
LpxC1	KP-1487	$6.8 \cdot 10^{-4}$ ($1.6 \cdot 10^{-5}$-$3.0 \cdot 10^{-2}$)
Meropenem	PA-UC12120	$2.0 \cdot 10^{-4}$ ($7.2 \cdot 10^{-6}$-$5.8 \cdot 10^{-3}$)

Human dose prediction

Table 3, Human dose prediction (mg).

		-1log kill after 24h		-2log kill after 24h		Stasis after 5 days	
Compound	Bacteria	Adap	Mut	Adap	Mut	Adap	Mut
MB1	PA-UC12120	145	105*	200	155*	420	70*
MB1	KP-1487	230	355	265	315	1255	485
MB2	KP-1487	490	675	560	765	2120	1010
LpxC1	KP-1487	3890	1145	5730	1460	3290	1315
Meropenem	PA-UC12120	230	255	300	560	500	2270

* Assuming no development of resistance.

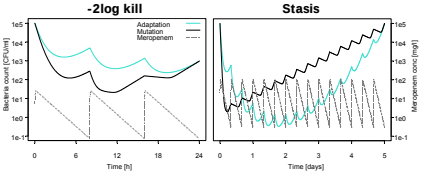

Figure 4, Predicted profile of PA-UC12120 with an inoculum of 10^5 CFU/ml and -2 log kill after 24 hours or stasis after 5 days. The PK profile of Meropenem shown is based on dose predicted by the mutation model.

Conclusions & Perspectives

- A tool box, including an adaptation model with an adaptive EC_{50} (including various adaptation functions, depending on concentration and/or time) and a mutation model (with mixture for the probability of mutation) was developed to analyze *in vitro* bacterial count-time profiles.
- Depending on the aim of the analysis and on the available data, the adaptation model (e.g. for rapid screening) or the mutation model (e.g. for more mechanistic insight) might be preferred.
- The human dose predicted by either modelling approach can be used to rank compounds for early stage decision making.

Find the poster here

References

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