Pharmacokinetic-Pharmacodynamic Modelling for Antibiotics: Static and Dynamic *In Vitro* Time-Kill Curve Experiments

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BACKGROUND and OBJECTIVES

In vitro time-kill studies are commonly conducted with the aim to assess the efficacy of antimicrobial agents. Developing a PK-PD model describing the time course of the effects may allow for an efficient use of these data and facilitate the comparisons between agents as well as making predictions under different settings possible.

Previously a general semi-mechanistic PK-PD model has been developed based on data from *in vitro* time-kill curve experiments with static antibiotic concentrations (1). The aim of the present study was to investigate the ability of the developed PK-PD model to predict and describe data from time-kill curve experiments with dynamic concentrations.

MATERIAL and METHODS

EXPERIMENTAL DESIGN

Bacteria: *Streptococcus Pyogenes* (M12 NCTC P1800)

Antibiotics: benzylpenicillin, cefuroxime, erythromycin, moxifloxacin, and vancomycin

Time-kill curve experiments:

- Static time-kill curve experiments were performed where a bacterial inoculum was exposed to constant antibiotic concentrations ranging from 0 to 64 times the MIC (1).
- Dynamic time-kill curve experiments were performed in an in vitro kinetic system (2) using a start concentration of 2 or 16 times the MIC with a simulated half-life of the drug (human or 1/3 of human, Figure 2).
- All experiments were performed at 35°C during 24 hours with frequent sampling for viable count. Each experiment was performed in at least duplicate and possible degradation of the drugs was monitored during the experiment.
- In total 187 experiments were included in the analysis (static n=135, dynamic n=52).

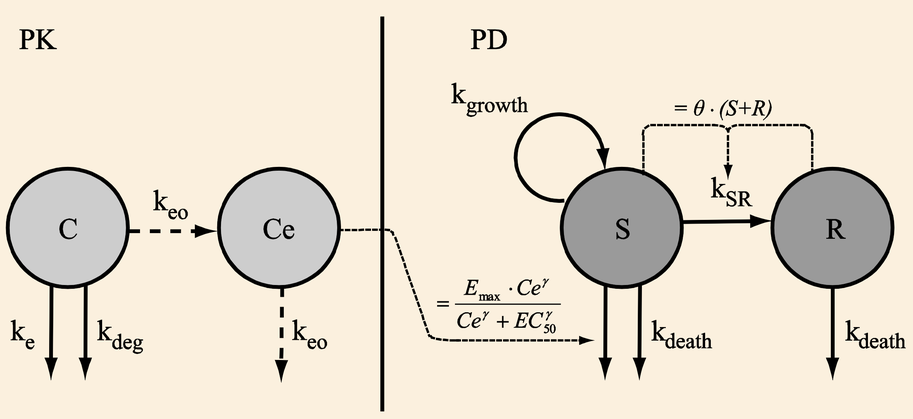

Figure 1. *Schematic illustration of the PK/PD model.*

The total bacterial population was divided into two subpopulations, one proliferating and drug susceptible population (S) and one resting and drug insensitive population (R). With this implementation the model successfully captures the low bacterial net growth and low antimicrobial efficiency when the system is reaching stationary phase. It also describes the early rapid killing effect as well as the presence of persisting cells often seen following antibiotic exposure.

DATA ANALYSIS

- The PK-PD model (Figure 1) developed based on static time-kill curve experiments was applied to the dynamic time-kill curve experiments.
- Observations from dynamic time-kill experiments were compared to model simulations (n=1000) based on parameters estimated using
  - a) only static data
  - b) only dynamic data
  - c) combined static and dynamic data.
- All data was modelled simulateously using NONMEM (version VI).
- Data below LOD (10 CFU/ml) was handled using the M3 method.
- An additive residual error on ln transformed data was used, and estimated as a replicate-specific error (ε_{repl}) and a common residual error (ε) using the L2 data item.
- The mixture module within NONMEM was used to allow for variability in the proportion of bacteria being in the resting state in the start inoculum.

REFERENCES

1. Nielsen EI, et al. Antimicrob Agents Chemother. 2007 Jan;51(1):128-36.
2. Löwdin E, et al. Antimicrob Agents Chemother. 1996 Nov;40(11):2478-82.

RESULTS

- Differences in experimental settings between the static and dynamic time-kill curve experiments (i.e. supply of fresh media, outflow of waste products, stirring) did not have a significant effect on the growth kinetics of the bacteria (Figure 3).

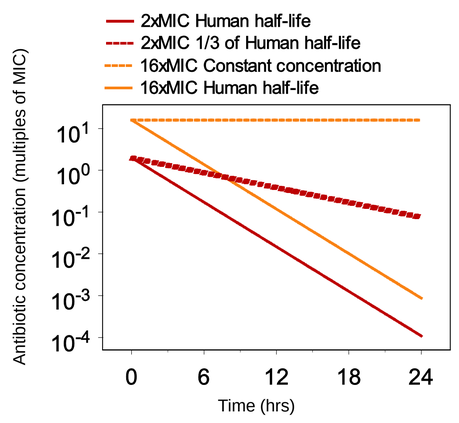

Figure 2. *Experimental design for the dynamic time-kill experiments*

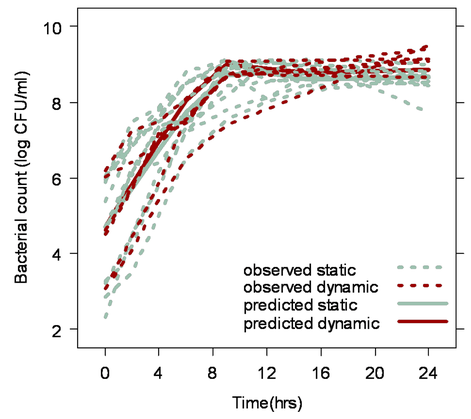

Figure 3. *Growth controls for static and dynamic time-kill experiments*

- For the majority of the antibiotics, the dynamic experiments was well predicted when applying the PK-PD model with parameter estimates based on the static experiments (Fig 4a). Further, when model parameters were re-estimated based on the dynamic experiments the observed bacterial counts in the dynamic experiments was very well predicted (Fig 4b). Adding data from dynamic experiments in the estimation, did improve the model fit for cefuroxime and vancomycin, indicating differences in sensitivity to experimental design between the antibiotics (Fig 4c).

4a. Estimation using only static data 4b. Estimation using only dynamic data 4c. Estimation using static and dynamic data

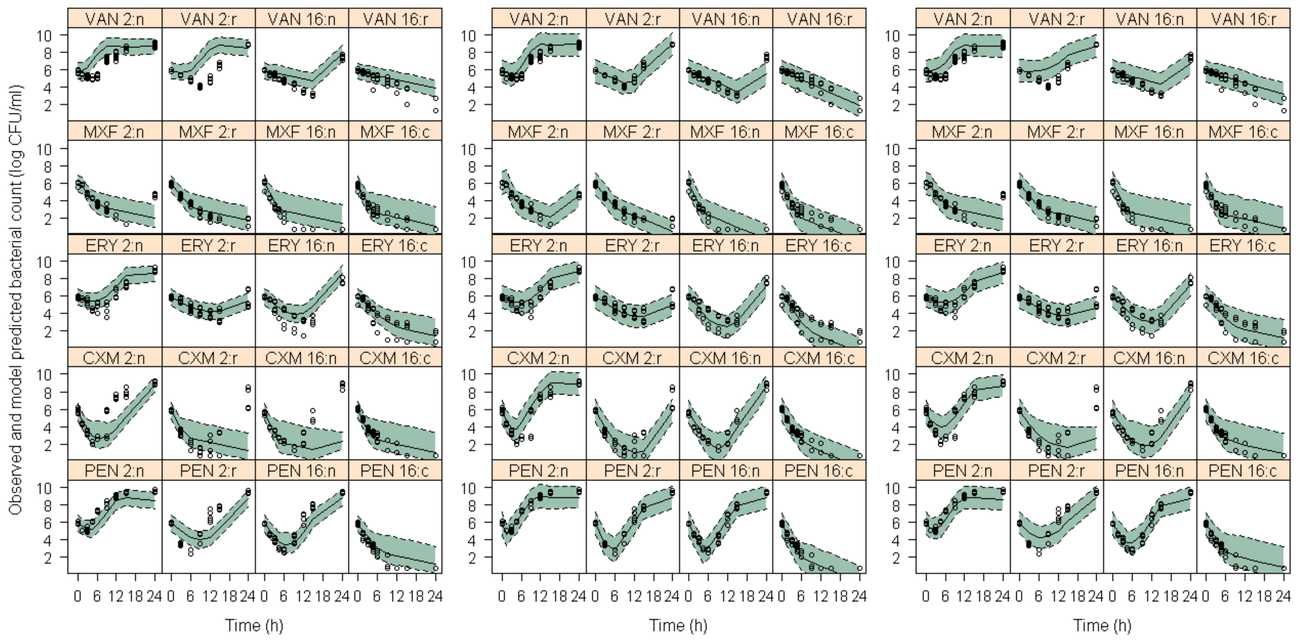

Figure 4. *Observed time-kill curves for the dynamic experiments with model predictions as median and 95% prediction interval.*

PEN benzylpenicillin, CXM cefuroxime, ERY erythromycin, MXF moxifloxacin, VAN vancomycin.
- 2:n C0=2xMIC, simulated human half-life
- 2:r C0=2xMIC, simulated 1/3 of human half-life
-16:n C0=16xMIC, simulated human half-life
-16:c C0=16xMIC, constant concentration in dynamic setting

- The PK-PD model with parameter estimates based on only the dynamic experiments resulted in relatively poor predictions of the results from the static experiments when the concentration of the antibiotic was close to the EC50. When using all data in parameter estimation, the model performance in predicting the static experiments were similar to when only the static data were included in the estimation (data not shown).

CONCLUSIONS

- For most antibiotics, the parameter estimates based on data from static time-kill curve experiments provided a good prediction of the data from dynamic experiments implying a limited need to perform labour intensive dynamic experiments.
- The structure of the previously developed PK-PD model could well describe also the data from dynamic time-kill curve experiments for all five investigated antibiotics.
- The PK-PD model allows an efficient summary of time-kill data and might provide a tool in optimized study design and in the development of improved dosing strategies not only for the studied drugs but also for other antibiotics.