

Mechanism-based Models for Growth and Killing of *Pseudomonas aeruginosa* by Tobramycin to Quantify and Predict the Inoculum Effect

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BACKGROUND

Published pharmacodynamic (PD) models for bacterial growth and killing by antibiotics cannot describe the time course of killing at various initial inocula (bacterial concentrations at time zero, CFU₀). Data from animal infection models show that high initial inocula increase mortality and require high antibiotic doses to achieve microbiological cure [1-3]. Mathematical models that can account for differences in bacterial inocula may be helpful to optimize antibiotic therapy and limit emergence of resistance.

OBJECTIVES

- 1) To develop a mechanism-based PD model that describes bacterial killing and growth of *P. aeruginosa* at various CFU₀.
- 2) To compare external validation with standard diagnostic plots for model qualification.
- 3) To predict benefits of dosing every 24h.

METHODS

Experimental

- Time kill experiments were performed in duplicate.
- Nine tobramycin concentrations from 0 to 64 mg/L
- *P. aeruginosa* (strain PAO1, MIC: 1 mg L⁻¹)
- Ca²⁺ and Mg²⁺ supplemented Luria-Bertani broth
- Initially at inocula of 10⁶ and 10⁹ CFU mL⁻¹ (CFU: colony forming units).
- Prospective validation at CFU₀ of 10^{7.5} CFU mL⁻¹.
- Samples (n=13) serially collected over 48h.

Modeling

- Literature model [4], one parameter set per CFU₀
- Compared to a new mechanism-based model with one parameter set for all inocula.
- Log-transformed CFU counts fitted in NONMEM® VI (level 1.2), LAPLACIAN method, additive error.
- YLO method for counts below 20 CFU/mL

RESULTS

The initial slope of Ln(CFU) vs. time curves was about five-times smaller at the same drug concentration for CFU₀ of 10⁹ compared to CFU₀ of 10⁶ (Fig 1).

The literature model required one parameter set per inoculum. The mechanism-based model yielded an 187 points better objective function and had three more parameters.

The dual mechanisms of drug action including a delayed drug effect of the new model (Fig. 2) better captured the concentration dependent lag-time of bacterial killing at both inocula shown in Fig. 3. The prospective validation was based on parameter estimates of CFU₀ of 10⁶ and 10⁹ CFU mL⁻¹.

The model predicted that a 6 h lag-time of bacterial killing with a nadir of bacterial counts at about 16 h would occur for a concentration of 0.5 mg L⁻¹ (Fig. 4). Experimental data showed this unusual time course of bacterial counts for a concentration of 1 mg L⁻¹. Dosing q24h was predicted to achieve the lowest nadir CFU counts (especially when using peripheral concentrations) and to achieve nadirs earliest (Fig. 5).

CONCLUSIONS

1. The new proposed model successfully described data at all studied inocula with one parameter set.
2. Incorporating a dual mechanism of action and a delayed drug effect significantly improved the ability of the model to describe the shape of the bacterial counts vs. time profiles across all three inocula.
3. Prospective validation was a valuable tool for model qualification.
4. Dosing q24h was predicted to be superior to shorter intervals for high and low initial inocula.

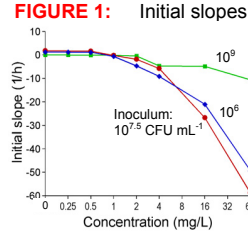


FIGURE 2: PD model for tobramycin vs. *P. aeruginosa*

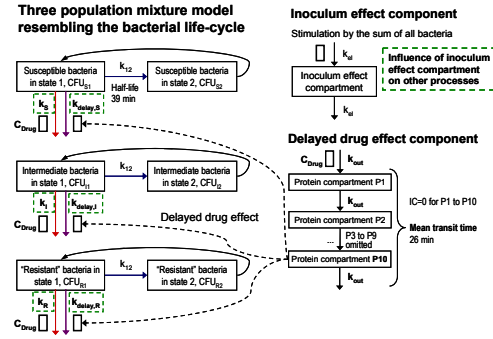
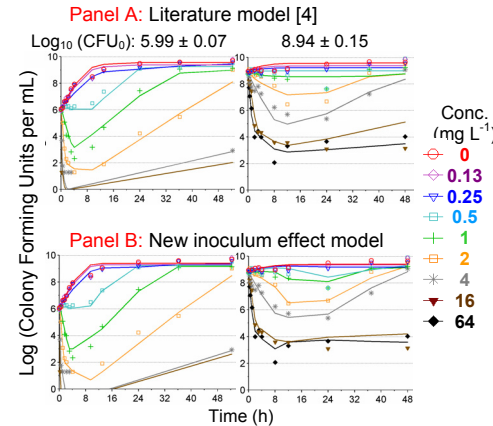


FIGURE 3: Curve fits for one replicate



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FIGURE 4: Prospective validation – simulated (left) and observed (right) CFU counts for an unstudied inoculum (10^{7.5} CFU mL⁻¹)

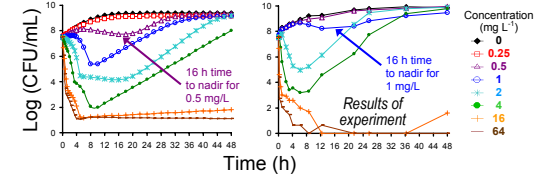
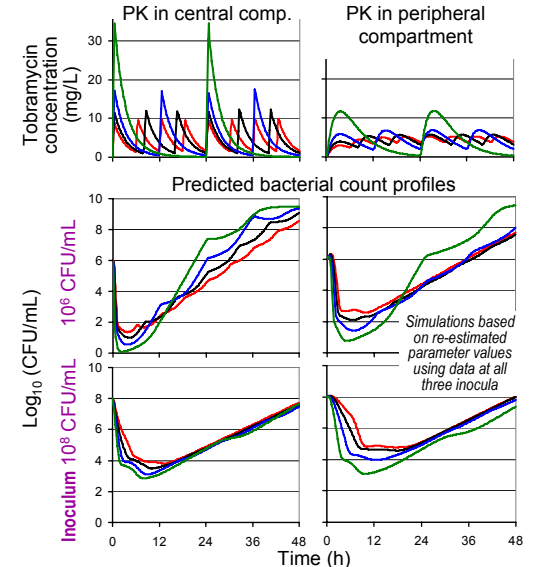


FIGURE 5: Comparison of dosing intervals (PK from ref. [5])

Concentrations for q24h, q12h, q8h, & q6h dosing (all: 10 mg/kg/day)



References

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