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A Novel Pharmacodynamic Model for Gatifloxacin vs. *Salmonella typhi* in Timed Kill Curves

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ABSTRACT

INTRODUCTION: Quinolones are the mainstay of treatment for *Salmonella typhi* (*S. typhi*) infections but there is growing concern about resistance to older quinolones & poor understanding of quinolone / *S. typhi* pharmacodynamics. We have developed a new approach to modeling kill curve data and have applied it to describing the pharmacodynamics of gatifloxacin vs. *S. typhi*.

METHODS: Log-phase cultures (10^{7-8} CFU/mL) of *S. typhi* (MIC=0.5 mg/L) were exposed to gatifloxacin at 0, 0.5, 1, 2, 4 & 8xMIC; bacterial counts (CFU) were obtained serially over 24h. Time-course of CFU was fit to a pharmacodynamic model with capacity-limited bacterial growth, 1st-order rate constant for death (Kd) & a Hill-type function in which gatifloxacin enhanced Kd. The total CFU was represented by a mixture model, with up to 4 sub-populations differing in gatifloxacin susceptibility. Each kill curve was fit individually & later simultaneously, using Adapt II. Akaike's Information Criterion was used to determine the number of sub-populations and which parameters would be allowed to vary between kill curves.

RESULTS: The final model had inter-kill curve variance in maximum velocity of growth & 4 sub-populations. The 1st 2 sub-populations were 99.99% of the initial CFU with sensitivities ≤ 1xMIC & the other 2 sub-populations were each < 0.01% of total CFU, with sensitivities of 2.3 & 4.4xMIC. The gatifloxacin Emax was a 17-fold increase in Kd. Goodness of fit was excellent with an overall r^2=0.96 (Observed=1.01*Fit-0.15).

CONCLUSIONS: This approach to pharmacodynamic modeling of *in vitro* data will give better insight into the activity of gatifloxacin vs. different *S. typhi* sub-populations & aid in determining regimens which minimize therapeutic failure due to the development of resistance.

INTRODUCTION

Typhoid fever is an acute infection caused by *S. typhi* ; a bacteria that causes over 16 million illnesses and 600,000 deaths/yr worldwide. Timely treatment and bacteria eradication reduces morbidity, mortality and the incidence of resistance. Mainstay of treatment now includes fluoroquinolones due to increasing resistance to traditional treatments such as SMP/TMX especially in developing countries. There is a need to optimize fluoroquinolone therapy when used, in order to minimize the development of resistance and optimize clinical outcome.

The use of pharmacodynamic models can help characterize the time course activity of drugs on bacterial growth and death. Modeling concentration vs. rate and extent of kill and/or re-growth using timed kill-curves can provide important insights into concentrations needed to optimize outcomes in man.

We have developed a novel mathematical model for characterizing bacterial rates of replication and death and for the effects of anti-infectives on this process. This model has been applied to the co-modelling of results of timed kill-curve experiments for gatifloxacin against *S. typhi*.

METHODS

- MIC to an isolate of *S. typhi* to gatifloxacin (MIC=0.5) was determined in triplicate following NCCLS criteria
- Timed kill-curves were done at concentrations ranging from 0.5 – 8xMIC
- Samples were taken at 0,1,2,4,5,and 24hrs to determine the bacterial CFU/mL.
- The Area under the CFU curve for each kill-curve (AUCFU) and the growth control (AUCGC) was computed using the numerical integration function of ADAPT II
- The Log10(AUCFU/AUCGC)ratio was plotted against the C/MIC and fit to a Hill type function
- The data was subsequently fit to candidate models assuming capacity limited rate of growth and the drug effect acting either by inhibition of bacterial replication or enhancement of death
- The total inoculum was represented as a mixture of 1-4 homogenous sub-populations which differed in percent of total initial inoculum and drug sensitivity
- The differential equation describing each bacterial sub-population is as follows (Model shown in Figure 1):

$$\partial CFU_i/\partial t = (VGmax * CFU_i)/(CFUm_i + CFU_{TOT}) - Kd * CFU_i$$

- Where CFU_i is the CFU/mL of the ith population, VGmax is the maximum velocity of growth (CFU/ml/hr), $CFUm_i$ is the CFU/mL associated with half the maximal growth
- CFU_{TOT} is the sum of all sub-populations combined and Kd is the first order rate constant for bacterial death (hr^{-1})
- All sub-populations were assumed to share a common VGmax, CFUm and Kd

METHODS CONT'D

- Drug effect was modeled as a Hill-type function that either decreased bacterial capacity-limited replication or enhanced 1st order death as follows:

$$I(i) = 1 \pm \ [Emax * [Drug]^H] \ / \ [STm_i{}^H + [Drug]^H]$$

- I(i) is multiplied by the replication term or the rate constant for death
- Where Emax is the maximum effect, H is Hill's constant of sigmoidicity, and STm_i is the serum inhibitory titer term associated with 50% inhibition of replication or enhancement of bacterial death.
- Each experiment was fit individually and later simultaneously using maximum likelihood & then MAP Bayesian estimation
- Simultaneous fitting was allowed to have up to 4 sub-populations
- Model discrimination was by AIC to determine the number of sub-populations and if CFUm or VGmax would be allowed to vary between experiments.

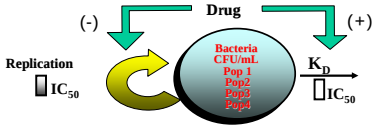

Figure 1: Pharmacodynamic model. CFU/mL, bacteria colony forming units/mL; Pop1-4, bacteria sub-populations; K_D, first-order bacterial death rate constant (hr^{-1}); IC_{50} drug concentration of 50% inhibition of growth or acceleration of death (μg/mL); Drug: Gatifloxacin.

Table 1. Model Parameter Estimates

Parameter	Value	Parameter	Value
VGmax1	1.28 x 10^9	H	19.9
VGmax2	7.59 x 10^8	STm1	0.0107
VGmax3	8.50 x 10^8	% POP 1	95.8%
VGmax4	2.51 x 10^8	STm2	0.993
VGmax5	7.64 x 10^8	%POP 2	4.22%
VGmax6	1.28 x 10^8	STm3	2.28
CFUm	9.77 x 10^8	%POP 3	0.0093%
T1/2min	0.038 h^{-1}	STm4	4.44
T1/2max	1.43 h^{-1}	%POP 4	0.0046%

VGmax1-6 is the VGmax for each experiment

RESULTS

- The maximal effect was a 4.76 Log difference in AUCFU compared to the AUCGC with a Hill's constant of 1.47 (Fig 2A)
- The C/MIC associated with 50% of Emax in Fig 2A was 0.56
- Drug effect was best modelled as enhancing Kdo with the model requiring 4 sub-populations
- The 1st and 2nd sub-populations made up ~99.99% of the total inoculums, with sensitivities of 0.01 and 0.99xMIC. The 3rd and 4th sub-population made up ~0.014% of the initial inoculum, with sensitivities of ~2 and 4xMIC
- Allowing for inter-experimental variance in VGmax across kill-curves provided a better fit than allowing inter-experimental variance in the CFUm
- The fit of the individual pharmacodynamic models to the kill curve data was excellent, median (range) r^2=0.985 (0.81-1). See Table 1 for model parameters and Figure 3 for plots
- The model fit the data with an overall r^2=0.96 (Observed=1.01xFit-0.15)

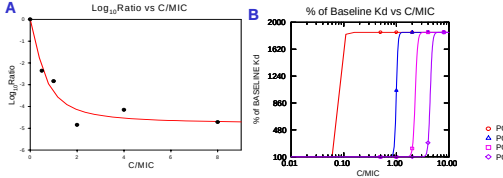

Figure 2: Log_{10}Ratio vs C/MIC (A) and % of Baseline Kd vs. C/MIC (B). Symbols represent observed data points, solid line represents model fits.

CONCLUSIONS

- The time course of gatifloxacin effect on *S. typhi* can be well co-modeled using the pharmacodynamic model
- Gatifloxacin can be best modelled as enhancing the rate constant of death
- A mixture of 4 homogenous sub-populations can explain the effect of low concentrations and inability of MIC to predict kill and eradication
- Concentrations that are ineffective for all the sub-populations could increase the probability of resistance development due to mutation or selection
- Drug effect is essentially a step function as is shown by the high value of the Hill's constant (H=19.9; in Table 1)
- The effect of concentration on Kd differs for each sub-population
- The maintenance of adequate concentrations against the most resistant sub-population is necessary to ensure eradication

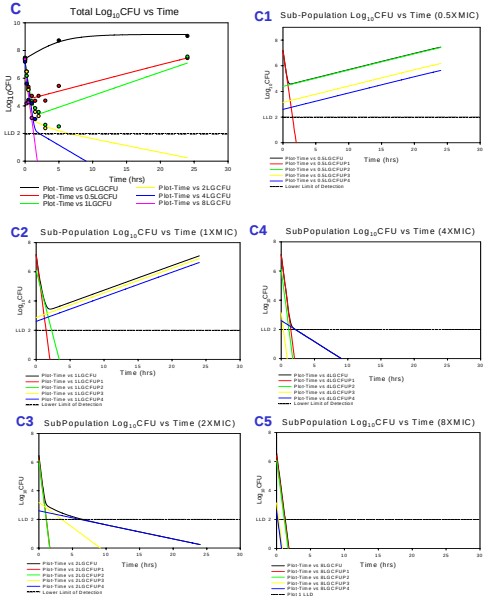

Figure 3: Model fits to time-kill curve data for gatifloxacin - all populations and C/MIC (C); Total and individual sub-populations for 0.5, 1,2,4, and 8XMIC (C1-C5). Symbols represent observed data points, solid line represents model fits.