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Use of an *in vitro* Pharmacodynamic Model & Monte Carlo Simulation to Characterize the Pharmacokinetics and Pharmacodynamics of Gatifloxacin vs *Salmonella* Typhi

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ABSTRACT

Salmonella enterica serotype Typhi and non-typohoidal *Salmonella* remain major causes of morbidity and mortality worldwide. Ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol no longer provide reliable coverage of *Salmonella* and fluoroquinoloes have emerged as first-line treatment options. Due to mounting evidence of decreased *in vitro* susceptibility and diminished clinical response to fluoroquinolone therapy, it has been suggested that the NCCLS breakpoints for the salmonellae be re-evaluated. We utilized an *in vitro* infection model to determine which pharmacokinetic-pharmacodynamic (PK-PD) measure was most closely linked to fluoroquinolone activity against salmonellae and the magnitude that was predictive of efficacy. Monte Carlo simulation was utilized to determine the probability of attaining potential susceptibility breakpoints for three fluoroquinolones. The free-drug AUC_{0-24}:MIC ratio was the PK-PD measure most predictive of efficacy and a ratio of 105 corresponded to 90% of maximal activity. Simulation results suggest susceptible breakpoints of 0.12 μg/mL for ciprofloxacin and gatifloxacin and 0.25 μg/mL for levofloxacin. These proposed breakpoints correspond to the MIC separating the wild-type susceptible organism population from those strains possessing single-step mutations in the quinolone resistance-determining region (QRDR). These results that integrate PK-PD measures and fluoroquinolone MIC distributions in the genetic context of examined *Salmonella*, clearly demonstrate the prudent use of a lower susceptibility breakpoint minimizes the probability of clinical failure or delayed response in fluoruqinolone-treated patients.

INTRODUCTION

- Over the last decade, fluoroquinolones have emerged as the mainstay of therapy for invasive infection associated with Typhi and non-typhoidal *Salmonella* serotypes. At the same time, the increasing incidence of infection with salmonellae resistant to nalidixic acid, which usually also display decreased susceptibility to fluoroquinolones, has raised considerable global concern.
- The vast majority of nalidixic acid-resistant strains remain within the current susceptible range for ciprofloxacin (≤ 1 μg/mL) as recommended by the NCCLS. However, the probability of clinical response to fluoroquinolone therapy in patients with invasive *Salmonella* infection is lower in those with nalidixic acid-resistant compared with –susceptible strains.

OBJECTIVES

- To identify the pharmacokinetics-pharmacodynamics (PK-PD) measure (i.e., free-drug (*f*) AUC_{0-24}:MIC, fC_{max}:MIC, or the duration of time free drug concentrations remain above the MIC (T>MIC)) that best predicts efficacy;
- To determine the magnitude of the PK-PD measure required for 90, 95, and 99% maximal efficacy;
- To utilize Monte Carlo simulation to integrate human pharmacokinetic data and PK-PD magnitude targets in an effort to determine MIC susceptibility breakpoints of ciprofloxacin (CIP), gatifloxacin (GAT), and levofloxacin (LEV), when testing salmonellae; and
- To correlate these measures with contemporary fluoroquinolone MIC population statistics.

MATERIALS AND METHODS

Bacteria, media, and susceptibility testing

- Two S. Typhi isolates selected for these experiments. Isolate 85-1416G had an elevated MIC vs fluoroquinolones of 0.25 - 0.5 μg/mL (GAT, 0.5 μg/mL) and strain G6/9 had a non-susceptible level MIC to fluoroquinolones (GAT, 4 μg/mL).
- The quinolone-resistance determining region (QRDR) for genes *parC, parE, gyrA* and *gyrB* were amplified by PCR.
- The in vitro PD model also used cation-adjusted Mueller-Hinton broth and quantitation of isolates was performed on MH agar plates.
- MIC population distributions were generated by the SENTRY Program using, validated broth microdilution methods for CIP, GAT, and LEV.

In vitro model and sample processing

- The one-compartment *in vitro* PD model used in these studies has been described previously.[1]
- An initial inoculum of 10^7 CFU/mL of the test strain was prepared from an overnight culture.
- Bacteria were exposed to changing GAT concentrations simulating a GAT half-life of 8 hours, similar to that observed in humans.[2] Once daily regimens were simulated to deliver steady state *f*AUC:MIC ratios ranging from 6 to 185.
- To confirm the simulation of human PK parameters, samples were collected throughout each model experiment.
- Quantitative cultures was assessed at –1, 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 hours. For twice daily regimens, quantitative cultures were also assessed at 12, 13, and 14 hours.
- The mean change in $\log_{10}$ CFU/mL was calculated for each duplicate study and time-kill curves were constructed by plotting $\log_{10}$ CFU/mL versus time.

Fluoroquinolone concentration determinations

- GAT concentrations were determined by a previously validated ion-paired high-performance liquid chromatography method assay.[2]

PK-PD analyses and Monte Carlo simulation

- Non-compartmental methods (WINNONLIN version 2.1, Pharsight Corp, Lexington, KY) and actual drug concentration data from each experiment were used to determine the following PK parameters: fC_{max}, elimination rate constant, half-life, and $fAUC_{0-24}$. The $fAUC_{0-24}$ was calculated using the trapezoidal method. Experimental PK and baseline GAT MIC data were used to determine the following PK-PD measures for each experiment: $fAUC_{0-24}$:MIC ratio, fC_{max}:MIC ratio, and %T>MIC.
- Drug effect was quantified by normalizing the 24 hour drug AUCFU by the growth control area, and taking the logarithm of the ratio as in Equation 1.

$$\text{Log ratio area} = \log_{10}\left[\frac{\text{AUCFU}_{(0-24)}}{\text{AUCFU}_{\text{Growth Control }(0-24)}}\right] \quad (1)$$

Log ratio values of zero indicate no drug effect, with larger negative values indicative of increasing drug effect. For example, a –1 log ratio implies 90% less area in the drug containing experiment over 24 hours compared to growth control. Using non-linear regression (Systat Software Inc., Version 10, Richmond, CA), a Hill-type model was fit to the log ratio of the AUCFU to obtain estimates of E_{max} (fitted maximum $\log_{10}$ reduction in bacteria), $fAUC_{0-24}$:MIC (the ratio required to achieve 50% of E_{max}), and H (Hill's constant, which accommodates sigmoidicity) (Equation 2).

$$\text{Log ratio} = -\left[\frac{\text{Emax} \bullet \left[f\text{AUC:MIC}_i\right]^H}{f\text{AUC:MIC}_{50i}{}^H + \left[f\text{AUC:MIC}_i\right]^H}\right] \quad (2)$$

- PK-PD target attainment analyses were carried out using Monte Carlo simulation. Dosing regimens modeled included GAT (400 mg once daily), CIP (500 mg twice daily), and LEV (500 mg once daily). Five thousand patient simulations were carried out using Crystal Ball, 2000.1 by Decisioneering, Inc. (Denver, Colo.) using the Equation 3.

$$f\text{AUC}_{0-24}:\text{MIC} = \frac{f \bullet \text{AUC}_{0-24}}{\text{MIC}} \quad (3)$$

PK from healthy subjects were obtained from FDA-approved product labels.[3,4,5,6] The mean (± standard deviation) AUC_{0-24} for GAT, CIP, and LEV was 34.4 (5.7), 28.2 (5.4), and 47.5 (6.7), respectively. The fraction of total drug bound to serum proteins was assumed to be 0.20, 0.30 and 0.31 for GAT, CIP, and LEV, respectively. The probability of attaining 90% E_{max} was estimated for each agent.

RESULTS

- **Figures 1 and 2** show mean PK-PD time-kill curves for GAT administered once daily for strains 85-1416G and G6/9. Strain 85-1516G had a single *GyrA* mutation, while strain G6/9 had 2 *GyrA* and 2 *ParC* mutations.
  - Irrespective of the number of QRDR mutations, similar patterns of bactericidal activity were observed.

Figure 1: Mean PK-PD time-kill curves of GAT administered once daily against a GAT-susceptible S. Typhi strain (85-1416G; MIC: 0.5 μg/mL)

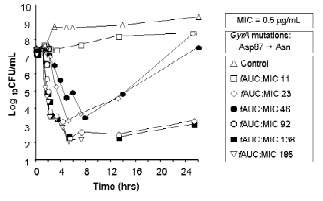
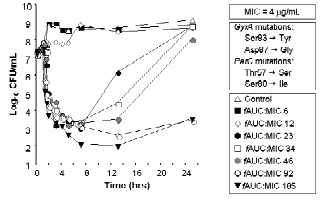
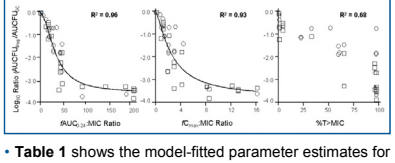


Figure 2: Mean PK-PD time-kill curves of GAT administered once daily against a GAT-resistant S. Typhi strain (G6/9, MIC: 4 μg/mL)



- **Figure 3** shows the relationship between exposure and response for three PK-PD measures. The square symbols (□) represent a strain with a GAT MIC value of 0.5 μg/mL, while the circle symbols (○) represent a strain with a GAT MIC value of 4 μg/mL.
  - $fAUC_{0-24}$:MIC and fC_{max}:MIC ratio best correlated with GAT bactericidal activity

Figure 3: Relationships between GAT exposure and response



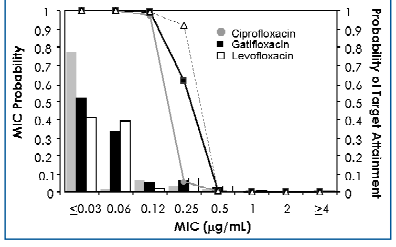
- **Table 1** shows the model-fitted parameter estimates for the $fAUC_{0-24}$:MIC and fC_{max}:MIC ratios.
  - For the $fAUC_{0-24}$:MIC ratio, the IC_{50} was 34.7 and 90% E_{max} was 105.
  - fC_{max}:MIC ratio, the IC_{50} was 2.61 and 90% E_{max} was 12.7.

Table 1: Model-fitted parameter estimates

Model-fitted parameter estimates	$fAUC_{0-24}$:MIC	fC_{max}:MIC
E_{max}	-3.58	-3.80
Hill's constant	2.04	1.39
IC_{50}	34.7	2.61
Percentage of E_{max}		
90	105	12.7
95	152	21.7
99	348	71.2

- **Figure 4** shows the fractional probability of PK-PD target attainment ($fAUC_{0-24}$:MIC ≥ 105). The MIC distribution was provided courtesy of the SENTRY Antimicrobial Surveillance Program (n=2,805) grey bars represent CIP, black GAT, and white LEV.
  - The fractional target attainment for CIP and GAT approached 1.0 for MIC values as high as 0.12 μg/mL and as high as 0.25 μg/mL for LEV.
  - These proposed breakpoints correspond to the MIC separating the wild-type susceptible population from those possessing single-step mutations in the QRDR.

Figure 4: Fractional probability of PK-PD target ($fAUC:MIC_{0-24}$ ≥105) attainment for CIP, GAT, LEV, and MIC histogram of CIP, GAT, and LEV against *Salmonella* spp.



CONCLUSIONS

- These data suggest that the current susceptibility breakpoints for fluoroquinolones published by the NCCLS may need to be altered to better predict clinical efficacy. Moreover, changing susceptibility breakpoints for fluoroquinolones will clearly negate the need for use of the nalidixic acid screening test, which is recommended by the NCCLS.

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