MONTE CARLO SIMULATION TO ESTIMATE *IN VITRO* SUSCEPTIBILITY BREAKPOINTS FOR MOXIFLOXACIN, GATIFLOXACIN, AND LEVOFLOXACIN AGAINST *STAPHYLOCOCCUS AUREUS*

A-136

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ABSTRACT

Background: Emerging resistance with staphylococci has called for a change in treatment paradigms and review of breakpoints. Current NCCLS breakpoints for gatifloxacin and levofloxacin against *Staphylococcus spp*. are ≤ 2 µg/mL for susceptible; breakpoints for moxifloxacin have not been established. Breakpoints can be evaluated using PK-PD models to predict *in vivo* efficacy.

Methods: Monte Carlo simulation was used to identify the probability of attaining PK-PD targets associated with efficacy using standard dosing regimens: moxifloxacin 400 mg QD, gatifloxacin 400 mg QD, and levofloxacin 500 mg QD. Single oral dose Phase I $AUC_{0-\infty}$ data for moxifloxacin was obtained (n=374), FDA submitted PK data were used for gatifloxacin and levofloxacin; all were adjusted for protein binding. PK-PD target was derived from a neutropenic murine-thigh model that identified a static AUC:MIC for reference quinolones. A target free-drug (*f*) 24hr AUC:MIC value of 30 was evaluated. Clinical isolate MIC distributions, including MSSA and MRSA, were obtained from surveillance studies. 5000 subject simulations were performed using fixed and continuous MIC data.

Results:

	PK-PD Target *f*AUC$_{24}$:MIC = 30				
	MIC (µg/mL)				
	0.25	0.5	1	2	Continuous Distribution*
% of Subjects Achieving PK-PD Target for moxi/gati/levo	100/100/100	93/100/100	8/23/74	1/1/1	73/66/62
Cumulative % MIC Distribution for moxi/gati/levo *	60/65/60	61/66/62	70/68/63	80/80/64	100/100/100

*N=3,204 strains for moxifloxacin; N=35,528 strains for gatifloxacin; and N=19,296 strains for levofloxacin

Target attainment rates correlated extremely well with *S. aureus* MIC population statistics; MIC_{50}/MIC_{90} for moxifloxacin, gatifloxacin, and levofloxacin are 0.06/4, 0.12/4, and 0.25/8 µg/mL, respectively. Probability of target attainment was ≥ 90% for MIC values ≤ 0.5 µg/mL for all regimens, but approached zero at a MIC value of 2 µg/mL. At a MIC value of ≤ 0.5 µg/mL, MRSA accounted for ~10% of susceptible strains.

Conclusion: Target attainment was similar for moxifloxacin, gatifloxacin, and levofloxacin. At a PK-PD target (*f*AUC_{24}:MIC) of 30, these data suggest a breakpoint for moxifloxacin, gatifloxacin, and levofloxacin of ≤ 0.5 µg/mL for susceptible. Correlation between *in vitro* tests and clinical outcome statistics is needed.

INTRODUCTION

- Resistance rates among Gram-positive organisms have increased dramatically over the past decade and staphylococci have emerged as one of the most prevalent pathogens in nosocomial infections [1,2].
- Inadequate treatment of infections caused by Gram-positive organisms has resulted in increased morbidity and mortality [3].
- Clinicians are often guided in choosing antimicrobial treatment by susceptibility breakpoints [4,5].
- Susceptibility breakpoints can be estimated using Monte Carlo simulation to integrate pharmacokinetic-pharmacodynamic (PK-PD) animal infection models, human PK, and *in vitro* microbiological activity data to predict clinical and microbiological response [6,7].
- For this analysis, Monte Carlo simulation was used to estimate susceptibility breakpoints for moxifloxacin, gatifloxacin, and levofloxacin against *S. aureus*.

INTRODUCTION (CONT.)

- At the time these analyses were conducted, the National Committee on Central Laboratory Standards (NCCLS) breakpoint for gatifloxacin and levofloxacin against *Staphylococcus spp*. was ≤ 2 µg/mL; a breakpoint for moxifloxacin had not yet been established [4,5].
- These data were presented to the NCCLS in June 2004 as decision-support for the establishment of staphylococcal susceptibility breakpoints for moxifloxacin and the re-evaluation of those breakpoints for other fluoroquinolones.

METHODS

PK-PD TARGET EXPOSURE

- A neutropenic murine thigh infection model was used.
- Five strains of *S. aureus* were evaluated and MIC values were determined by the broth microdilution method described by the NCCLS [4,5].
- Mice were inoculated with 10^6 to 10^7 CFU/mL 2 hours prior to moxifloxacin administration by injection of 0.1 mL of inoculum into each posterior thigh.
- Single-dose serum pharmacokinetic studies were performed in thigh-infected mice given subcutaneous doses of moxifloxacin (0.293 to 75 mg/kg every 12 hours).
- For each of the examined doses, three mice were sampled at 24 hours after the start of treatment. Control mice were sampled at 0-hour and at 24 hours. Serial dilutions of thigh homogenates were plated for CFU determinations.
- Serum moxifloxacin concentrations were determined by standard microbiologic assays with *S. aureus* ATCC 6538p as the test organism and antibiotic medium 1 as the agar diffusion medium.
- The lower limit of detection for assays was 0.1 µg/mL with an intraday variation less than 14%.
- PK parameters were calculated using standard non-compartmental techniques.
- Serum protein binding in infected neutropenic mice was performed with ultra-filtration methods [8].
- Efficacy was calculated by subtracting the $\log_{10}$ CFU/thigh of each treated mouse at the end of therapy from the mean $\log_{10}$ CFU/thigh of control mice just prior to treatment (0 hour) and at the end of therapy (24 hour).
- A sigmoid dose-effect model was used to analyze the data:
  - $E = (Emax \bullet D^h)/(ED_{50}^N + D^h)$, where E is the effect, Emax is the maximal effect, D is the 24 hour total dose, ED_{50} is the dose required to achieve 50% Emax, and N is the slope of the dose-effect curve.
- The correlation between efficacy and three PK-PD indices (*f*$AUC_{0\text{-}24}$:MIC ratio, *f*peak:MIC ratio, and T>MIC) were examined by using nonlinear least-squares multivariate regression.

PHARMACOKINETIC PARAMETERS

- Moxifloxacin PK data ($AUC_{0-\infty}$) were from single-dose Phase I studies of the oral administration of moxifloxacin 400 mg to normal adult volunteers (n=374) (**Table 1**).
- Phase I single-dose $AUC_{0-\infty}$ values for orally administered gatifloxacin 400 mg daily and levofloxacin 500 mg were obtained from each product's label (**Table 1**) [9,10].
- Sample distribution of the moxifloxacin $AUC_{0-\infty}$ data was fit to a lognormal distribution.
- A lognormal distribution was also employed for gatifloxacin and levofloxacin PK data.
- The free fraction (*f*) of moxifloxacin, gatifloxacin, and levofloxacin were fixed at 0.61, 0.8, and 0.69, respectively.

METHODS (CONT.)

Table 1: Pharmacokinetic parameter values for moxifloxacin, gatifloxacin, and levofloxacin

Agent	Dose (mg)	$AUC_{0-\infty}$[a] (mg · hr/L)	Protein binding (%)
Moxifloxacin	400 mg q.d.	36.1 ± 9.1	37-50%[b]
Gatifloxacin	400 mg q.d.	33.0 ± 6.2	20%
Levofloxacin	500 mg q.d.	47.9 ± 6.8	24-38%[c]

[a] Mean and standard deviation (SD) values are from single oral dose healthy adult Phase I studies
[b] Moxifloxacin protein binding was fixed at 39%
[c] Levofloxacin protein binding was fixed at 31%

MICROBIOLOGICAL SUSCEPTIBILITY DATA

- Moxifloxacin, gatifloxacin, and levofloxacin susceptibility data were obtained from the SENTRY Antimicrobial Surveillance Program (2000-2002).
- Isolates were primarily from patients with documented pneumonia or bloodstream infections.
- MIC values were determined by the broth microdilution method described by the NCCLS [4,5].

MONTE CARLO SIMULATION AND SUSCEPTIBILITY BREAKPOINT ESTIMATION

- PK-PD target attainment analyses were performed using Monte Carlo simulation.
- Five thousand random subject simulations were performed using the following structural model:
  - *f*$AUC_{0\text{-}24}$:MIC = $f \cdot AUC_{0-\infty}$:MIC
- The MIC susceptible breakpoint was determined as the highest clinically relevant MIC value with a probability of PK-PD target attainment of 0.9.

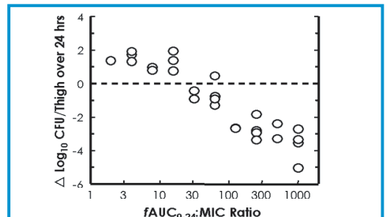
RESULTS

PK-PD TARGET EXPOSURE

- MIC values of moxifloxacin for the five strains of *S. aureus* ranged from 0.03 to 0.06 µg/mL:
  - Three strains were oxacillin-susceptible (MIC ≤ 2 µg/mL)
  - Two strains were oxacillin-resistant (MIC ≥ 4 µg/mL)
- The PK of moxifloxacin in infected neutropenic mice at doses of 4.68, 18.8, and 75 mg/kg was linear:
  - The elimination half-life was 0.6 to 0.8 hours
  - Mean peak serum concentration/dose ratio was 0.5 to 0.6
  - Mean AUC_{0-24}/dose ratio was 0.7 to 0.8
  - Serum protein binding was 50 to 55% at concentrations ranging from 1.0 to 5.0 µg/mL
- Mice had 6.79 ± 0.29 $\log_{10}$ CFU/thigh of *S. aureus* at the initiation of therapy and organisms grew 1.75 ± 0.34 $\log_{10}$ CFU/thigh in untreated control mice.
- Increasing moxifloxacin doses resulted in concentration-dependent killing.
- The highest dose studied reduced the bacterial burden at 0-hour by 3.12 ± 0.37 $\log_{10}$ CFU/thigh.
- A strong relationship was seen when results were correlated with the *f*$AUC_{0\text{-}24}$:MIC ratio, with an r^2 value of 89% (**Figure 1**).
- An *f*$AUC_{0\text{-}24}$:MIC ratio of approximately 30 is required for stasis.

RESULTS (CONT.)

Figure 1: Relationship between *f*$AUC_{0\text{-}24}$: MIC ratio and change in bacterial density of five strains of *S. aureus* in the thighs of neutropenic mice after 24 hours of moxifloxacin therapy

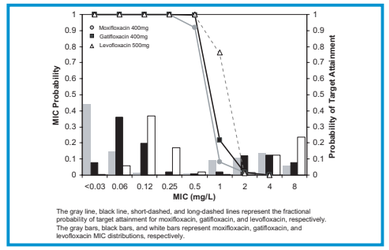


The dashed line represents the number of bacteria at the beginning of therapy. Positive values on the y-axis reflect bacterial growth, while negative values reflect bacterial death.An *f*$AUC_{0\text{-}24}$:MIC ratio of approximately 30 is required for stasis.

MICROBIOLOGICAL SUSCEPTIBILITY DATA

- **Figures 2**, **3**, and **4** show the MIC distribution for the three fluoroquinolones against all *S. aureus* isolates, oxacillin-susceptible *S. aureus*, and oxacillin-resistant *S. aureus*, respectively.
- The total number of isolates tested for moxifloxacin, gatifloxacin, and levofloxacin were 3,204, 35,528, and 19,296, respectively.
- The rank order of *in vitro* potency (most active to least) was as follows: moxifloxacin ($MIC_{50/90}$: 0.06/4 µg/mL); gatifloxacin ($MIC_{50/90}$: 0.12/4 µg/mL); and levofloxacin ($MIC_{50/90}$: 0.25/≥8 µg/mL).
- Approximately 8 to 10% of oxacillin-resistant isolates had fluoroquinolone MIC values of ≤ 0.5 µg/mL and 6 to 7% of oxacillin-susceptible isolates had MIC values of ≥ 1 µg/mL.

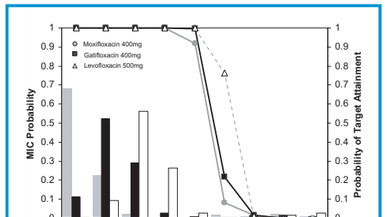
Figure 2: Fractional probability of PK-PD target attainment (*f*AUC_{24}:MIC = 30) for moxifloxacin, gatifloxacin, and levofloxacin against *Staphylococcus aureus*



The gray line, black line, short-dashed, and long-dashed lines represent the fractional probability of target attainment for moxifloxacin, gatifloxacin, and levofloxacin, respectively. The gray bars, black bars, and white bars represent moxifloxacin, gatifloxacin, and levofloxacin MIC distributions, respectively.

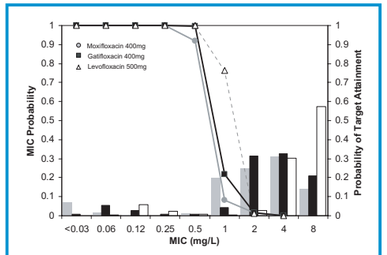
RESULTS (CONT.)

Figure 3: Fractional probability of PK-PD target attainment (*f*AUC_{24}:MIC = 30) for moxifloxacin, gatifloxacin, and levofloxacin against oxacillin-sensitive *Staphylococcus aureus*



The gray line, black line, short-dashed, and long-dashed lines represent the fractional probability of target attainment for moxifloxacin, gatifloxacin, and levofloxacin MIC distributions, respectively.

Figure 4: Fractional probability of PK-PD target attainment (*f*AUC_{24}:MIC = 30) for moxifloxacin, gatifloxacin, and levofloxacin against oxacillin-resistant *Staphylococcus aureus*



The gray line, black line, short-dashed, and long-dashed lines represent the fractional probability of target attainment for moxifloxacin, gatifloxacin, and levofloxacin MIC distributions, respectively.

RESULTS (CONT.)

MONTE CARLO SIMULATION AND SUSCEPTIBILITY BREAKPOINT ESTIMATION

- The forecast AUC distributions (mean ± SD) for moxifloxacin, gatifloxacin, and levofloxacin were 35.9 ± 9.04, 33.0 ± 6.1, and 48.1 ± 6.77 mg•hr/L, respectively.
- **Figure 2** shows the fractional PK-PD target (*f*$AUC_{0\text{-}24}$:MIC ratio ≥ 30) attainment over the *S. aureus* MIC distributions for each drug. **Figures 3** and **4** show the fractional target attainment over the oxacillin-sensitive and -resistant *S. aureus* distributions, respectively.
- The probability of target attainment was greater than 0.9 for MIC values ≤0.5 µg/mL for moxifloxacin, gatifloxacin, and levofloxacin regimens.
- Target attainment rapidly degraded for MIC values > 0.5 µg/mL and approached zero for MIC values ≥ 2 µg/mL.
- These data suggest susceptible MIC breakpoints of 0.5 µg/mL for moxifloxacin, gatifloxacin and levofloxacin.
- The estimated susceptibility breakpoints correlate extremely well with the SENTRY Antimicrobial Surveillance Program MIC distributions of *S. aureus*, including both oxacillin-susceptible and -resistant strains (**Figures 2, 3,** and **4**) [2].
  - Each fluoroquinolone displayed a bimodal distribution of MIC values, with a natural cleave or breakpoint at 0.5 µg/mL for all three agents.
  - This breakpoint effectively differentiated the oxacillin-susceptible and oxacillin-resistant *S. aureus* subpopulation as well as the quinolone-susceptible and -resistant subpopulations, the latter with QRDR mutations.

CONCLUSIONS

- The probability of target attainment at an MIC of ≤ 2 µg/mL, the previously establish NCCLS breakpoints, approached zero for all three studied fluoroquinolones using traditional dosing regimens.
- Results from this PK-PD analysis were presented to the NCCLS in June 2004 to establish a susceptibility breakpoint for moxifloxacin at ≤ 0.5 µg/mL, and to lower the susceptibility breakpoints for gatifloxacin and levofloxacin to ≤ 0.5 µg/mL and ≤ 1 µg/mL, respectively.
- The revised breakpoints resulted in nearly identical perceived spectrums of anti-staphylococcal activity as measured by percentage susceptible rates for all evaluated fluoroquinolones, and a clear lack of potency for the three agents against oxacillin-resistant *S. aureus* isolates.

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