Poster A1-1941
49th ICAAC
San Francisco, CA, USA
September 12-15, 2009

Pharmacokinetics and Pharmacodynamics of Delafloxacin in *S. aureus* Murine Thigh Infection Models

E. Burak[1], E. Bortolon[1], D. Molstad[1], Y. Wu1, H. Jing[1] and D. Girard[2]

[1]Rib-X Pharmaceuticals, Inc., New Haven, CT, USA and [2]Independent Consultant, Salem, CT, USA

Rib-X PHARMACEUTICALS
New Haven, CT 06511
Phone: 203-848-6923
Fax: 203-624-5627
http://www.Rib-X.com
Email: eburak@Rib-X.com

ABSTRACT

Background: Delafloxacin (DFX) is an investigational fluoroquinolone (FQ) active against a variety of Gram-positive bacteria, including methicillin- and quinolone-resistant strains of *Staphylococcus aureus* (MRSA, QRSA). DFX has demonstrated clinical efficacy in three Phase 2 studies; including complicated skin (cSSSI). Literature data for FQs indicate that the ƒAUC/MIC targets for *S. aureus* (SA) are considerably higher than for other gram-positives; therefore, our initiative focused on determining the PK/PD indices (PDI) for DFX against several SA strains using the mouse neutropenic thigh infection model.

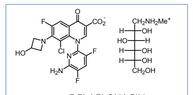
Methods: MICs were determined using CLSI methodology. Murine thigh infections were established in neutropenic CD-1 mice, subcutaneous therapy was initiated 2 hr following challenge covering a 20 to 512-fold dosage range using a dose-fractionation paradigm. The bacterial burden in tissues was determined 24 hours after therapy using standard techniques. Single-dose PK studies were conducted in infected mice and plasma levels determined using an LC/MS/MS assay. The 24-hour PDIs were estimated from PK parameters corrected for free fraction using non-compartmental methods. The correlation between efficacy and each of the PK/PD parameters was determined by nonlinear least-squares multivariate regression (E_{max} model).

Results: Delafloxacin demonstrated linear pharmacokinetics over the dosage range used in these studies. For the five SA strains 86-99% of the variance in the data can be explained by the ƒAUC/MIC of DFX. The median (range) ƒAUC/MIC PDI targets for DFX against the SA strains are: 9.3 (2.0-12.8) for stasis and 14.3 (6.3-18.5) for a 1-log kill.

Conclusions: DFX demonstrated in vivo activity against SA including QRSA. The PK/PD parameter that was most closely linked to outcome for DFX vs. SA was the ƒAUC/MIC. The ƒAUC/MIC for stasis and 1-log kill for DFX vs. SA was 9.3 and 14.3.

INTRODUCTION

Delafloxacin (DFX) is an investigational antibiotic within the fluoroquinolone class distinguished by good clinical safety, unusual physical properties, and excellent antibacterial activity against gram-positive organisms including both methicillin-susceptible *S. aureus* and MRSA (including quinolone-resistant strains). The chemical name of delafloxacin is 1 deoxy-1-(methylamino)-D-glucitol, 1-(6-amino-3, 5-difluoro-2-pyridinyl)-8-chloro-6-fluoro-7-(3-hydroxy-1-azetidinyl)-4-oxo-1,4-dihydro-3-quinolinecarboxylate (salt). The structure of the methylglucamine salt is shown on the right.



DELAFLOXACIN

DFX provides antimicrobial coverage of prevalent MRSA and methicillin-resistant coagulase-negative staphylococci. In general, the in vitro antibacterial activity of delafloxacin is more potent than that of levofloxacin and ciprofloxacin against most quinolone susceptible pathogens, including species responsible for surgical site infections, community and nosocomial respiratory tract infections, urinary tract infections, blood stream infections, skin and skin structure infections, and anaerobic infections.

Delafloxacin was the most active quinolone against quinolone-susceptible and -resistant *S. aureus*, MRSA, *S. epidermidis*, methicillin-resistant *S. epidermidis*, vancomycin susceptible or -resistant enterococci, viridans group streptococci, *S. pyogenes*, and *S. pneumoniae*. A diverse set of world-wide methicillin-resistant *S. aureus* isolates (MRSA) was evaluated and delafloxacin had minimum inhibitory concentration required to inhibit the growth of 90% of organisms (MIC_{90}) values of 0.5 µg/mL, while levofloxacin had MIC_{90} values >32 µg/mL.[1] DFX was more active or equivalent to the other quinolones against quinolone-susceptible and -resistant *Escherichia coli* and *Klebsiella* species as well as quinolone-susceptible *Pseudomonas aeruginosa*.[2]

Over 1400 subjects have received the oral formulation of delafloxacin in Phase 1 and Phase 2 clinical trials. DFX was most recently shown to be efficacious in a Phase 2 clinical study against complicated skin and structure infections (cSSSI).[3] In this trial, DFX clinical outcome was comparable to that of tigecycline while demonstrating a potential safety/tolerability advantage.

The purpose of this study was to evaluate the pharmacokinetic and pharmacodynamic profiles of DFX in the murine thigh infection model with various strains of *S. aureus* (both methicillin-susceptible and –resistant). This model has most recently been used to determine the PDI for other quinolones such as garenoxacin and gatifloxacin.[3-4]

METHODS

Microorganisms

The minimum inhibitory concentration (MIC) of delafloxacin was determined for all the strains used in this study using the microdilution method as outlined by the CSLI guidelines using cation-adjusted Mueller-Hinton broth.

The *S. aureus* isolates that were used in this study were:

Strain	MIC (mg/L)			
	Delafloxacin	Oxacillin	Levofloxacin	Clindamycin
S. aureus MSSA Smith	0.004	0.5	0.125	0.125
S. aureus MSSA ATCC 29213	0.006[a]	0.5	0.25	0.125
S. aureus MRSA 2926	0.016	>8	0.25	>8
S. aureus MRSA 11540	0.8[a]	>8	>8	0.125
S. aureus MRSA 11512	0.5	>8	16	ND[b]

[a]Geometric means of replicates, [b]ND – Not Determined

Drug Formulation

A stock of 40 mg/mL DFX was prepared in vehicle (30% hydroxypropyl-ß-cyclodextrin and 6% xylitol in 25mM meglumine; pH 8.4). DFX and vehicle were diluted 1:5 in 0.9% saline.

Protein Binding

Protein binding was determined with [^{14}C] DFX utilizing an equilibrium dialysis method. For mouse the average plasma protein binding was 97% and for human 84%.

Murine Thigh Infection Model

Six-week-old specific pathogen free female CD-1 mice (weight, 22-26g; Charles River, Portage, MI) were used for all studies. Neutropenia was rendered by injection of 150 and 100 mg/kg cyclophosphamide I.P. on Days -4 and -1, respectively. The inoculum for *S. aureus* 11540, *S. aureus* 2926, and *S. aureus* Smith was prepared as follows: bacteria were streaked from frozen stocks on to Blood agar (Tryptic Soy Agar + 5% Sheep Blood, BD #221261). 16-18 hrs prior to inoculation, three colonies from the freshly grown plate were added to 10 mL of cation-adjusted Mueller-Hinton (MH) broth and serially diluted 1:10, 10 times in succession into 10 mL of MH. The diluted cultures were then grown overnight with shaking at 35°C. After overnight incubation, the OD_{580} (optical density at at λ=580 nm) was measured for each dilution, and the dilution closest to OD_{580} = 0.3 was selected and further diluted 1:10 into pre-warmed MH broth. This culture was then placed in an incubator for 1.5 hr at 35°C with shaking to obtain logarithmic growth and diluted 1:10 prior to inoculation. The final inoculum CFU/mL density was confirmed by serial dilution and culture on Blood agar. For thigh infections, each mouse received 0.1 mL of the inoculum in the both caudal thigh muscles under light anesthesia (Isoflurane, Baxter).

RESULTS

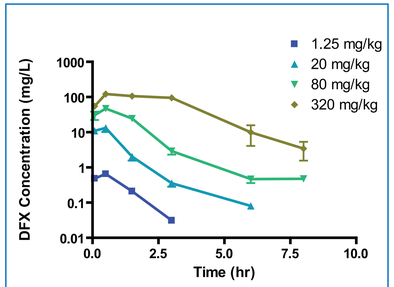


Figure 1. Pharmacokinetics of DFX following SC administration to mice at various doses.

Table 2. DFX Pharmacokinetic Parameters Following Administration at Various Doses.

Dose (mg/kg)	AUC_{total} 0-24 (mg*h/mL)	AUC_{free} 0-24 (mg*h/mL)	$Cmax_{total}$ (mg/mL)	$Cmax_{free}$ (mg/mL)	$T_{1/2}$ (h)
1.3	0.81	0.024	0.65	0.02	0.6
20	13.6	0.408	13.3	0.399	1.0
80	72.6	2.18	46.9	1.41	0.8
320	433.6	13	121.6	3.65	1.0

1) The C_{max} of DFX was approximately linear over the dose range tested whereas AUC had non-linear tendencies above 20 mg/kg.

2) AUC_{free}/MIC best described data obtained in the murine neutropenic thigh model. In general, the fits for the individual strains were far superior to combined data with R^2 values ≥ 86% (data not shown).

3) $\%T_{free}$>MIC for combined data showed a slight correlation with reduction in bioburden; however, regression analysis for certain individual strains did not converge.

4) $C_{max,\ free}$/MIC was not correlative with the reduction in bioburden.

METHODS CONT.

Pharmacokinetics

The single-dose pharmacokinetics of DFX, dosed subcutaneously (0.2mL/dose), were performed in thigh-infected mice at 1.25, 20, 80, and 320 mg/kg in neutropenic CD 1 mice. Each dose was administered two hours post-inoculation. For each subcutaneous dose examined, three mice per time point were sampled for the following time points: 0.5, 1, 2, 4, 6, and 8 hrs. Blood was collected via cardiac puncture under CO2 narcosis and placed in K2-EDTA vials. Plasma samples were analyzed for drug concentration by LC/MS/MS. A triple quadrupole mass spectrometer, Waters Quattro Micro, was used for MS analysis with electrospray positive ionization. Acetonitrile was used for protein precipitation of the samples. The linear range of the method is 0.010 to 10.0 µg/mL.

The following pharmacokinetic parameters were determined for each subcutaneously administered dose using Pharsight WinNonlin (v. 5.2). Parameters including area under the concentration time curve (AUC), maximum plasma concentration (C_{max}), time to achieve maximum plasma concentration (T_{max}), and the terminal elimination half-life ($T_{1/2}$) were determined via noncompartmental analysis.

The AUC, C_{max}, and T>MIC for doses given b.i.d. or q.i.d were estimated using non-parametric superposition in WinNonlin. Linear interpolation between measured parameters was used to estimate the AUC, C_{max}, and T>MIC, for the doses given between the measured doses.

PK/PD Index (PDI) Determination

Each group was sacrificed 26 hours after inoculation. Untreated control mice were sacrificed immediately after inoculation (T=0h), at the start of therapy (T=2h), and 24 hrs after the initiation of treatment (T=26). At the specified time points, mice were euthanized by CO_2 exposure and had the caudal thigh muscles harvested and individually homogenized in sterile bags. Serial dilutions of the homogenate were plated on Blood agar and incubated overnight for enumeration of the bacterial density.

Data analysis for PDI determination

The change in colony forming units was defined as the change in bacterial density after 24 hours of treatment compared to bacterial density at the onset of treatment (T=2h). To determine the PDI which best correlates with efficacy, plots describing the change in $\log_{10}$ CFU/thigh versus a) the 24 hour area under the free drug concentration over the MIC ($24h_{free}$AUC/MIC), b) the maximum free drug plasma concentration over the MIC ($C_{max\ free}$/MIC), and c) the percent of time the free drug concentration remains above the MIC ($\%T_{free}$/MIC) were constructed. Nonlinear least-squares multivariate regression was used to determine the correlation between efficacy and each of the above mentioned PK/PD parameters. The coefficient of determination, R^2, will be used to estimate the variance that could be due to regression with each of the PK/PD parameters.

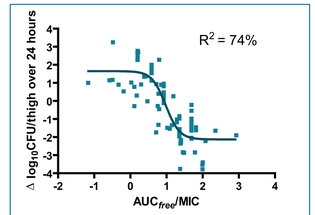


Figure 2. Change in bacterial burden as a function of AUC_{free}/MIC with five strains of *S. aureus*

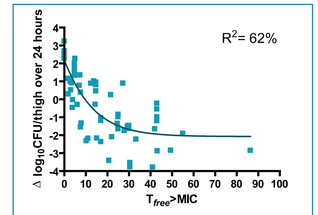


Figure 3. Change in bacterial burden as a function of %T>MIC with five strains of *S. aureus*

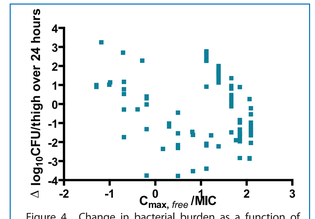


Figure 4. Change in bacterial burden as a function of Cmax/MIC with five strains of *S. aureus* (Non-linear regression did not converge)

Table 3. Free AUC/MIC Values for Bacterial Stasis and 1-$\log_{10}$ reduction in colony forming units for five Strains of *S. aureus*.

S. aureus strain	MIC (mg/L)	Free-drug AUC : MIC ratios associated with efficacy: Net bacterial stasis	Free-drug AUC : MIC ratios associated with efficacy: 1-$\log_{10}$ CFU ↓
ATCC 13709	0.004	3.8	7.6
11540 USA300	0.8	9.3	14.3
2926 USA 100	0.016	2	6.3
ATCC 29213	0.006	12.8	18.5
11512	0.8	9.5	17.9
	Median	9.3	14.3
	Min, Max	2.0, 12.8	6.3, 18.5

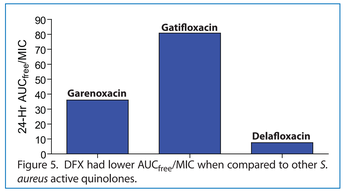


Figure 5. DFX had lower AUC_{free}/MIC when compared to other *S. aureus* active quinolones.

CONCLUSIONS

1) AUC/MIC was the best pharmacodynamic indicator for Delafloxacin against *S. aureus*

2) The AUC_{free}/MIC was consistent across 5 strains (ranging from 2 to 12.8 for stasis) with a wide range of MICs

3) AUC_{free}/MIC is lower than report for quinolones such as gatifloxacin and gemifloxacin

4) The AUC_{free}/MIC was used, in part, to aid dose justification for Phase 2/3 clinical trials

REFERENCES

1. Burak, E., Hopkins, S., Pillar, C., and Lawrence, L. In Vitro Activity of Delafloxacin Against Methicillin-Resistant Staphylococcus aureus in the United States, Europe and Asia. 19th ECCMID (2009), Helsinki, Finland, P-1080.
2. O'Riordan, W., Babazadeh, S., Keech, R., Manos, P, Mascola, M., Mehra, P., Surbec, J., Hopkins, S., and Burak, E. Results of a Phase 2 Study in Comparing Two Doses of Delafloxacin to Tygecycline in Adults with Complicated Skina and Skin Structure Infections. 19th ECCMID (2009), Helsinki, Finland, P-1794.
3. Andes D. Pharmacodynamics of the New Des-F(6)-Quinolone Garenoxacin in a Murine Thigh Infection Model. Antimicrob. Agents Chemother. 47:3935-3941, 2003.
4. Andes D. Pharmacodynamics of the New Fluoroquinolone Gatifloxacin in Murine Thigh and Lung Infection Models. Antimicrob. Agents Chemother. 46:1665-1670, 2002.