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Pharmacodynamics of the Fluorocycline TP-271 in a Neutropenic Murine Lung Infection Model with Methicillin-resistant *Staphylococcus aureus*

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Abstract

Background: TP-271 is a novel broad-spectrum fluorocycline being developed by Tetraphase Pharmaceuticals to combat respiratory disease caused by bacterial biothreats and antibiotic-resistant pathogens. This study was performed to determine the pharmacodynamic parameter and magnitude for TP-271 that is best predictive of efficacy in lung infections caused by methicillin-resistant *S. aureus* (MRSA).

Methods: Female CD-1 mice were rendered neutropenic by IP injection of Cytoxan (150/100 mg/kg on days -4/-1 pre-infection). Infection was established by intranasal instillation of 10e7 colony forming units for three HA-MRSA strains expressing multiple resistance mechanisms (tetracycline-, fluoroquinolone- and macrolide-resistant). TP-271 was administered IV at 2 hours post-infection and lung CFUs determined at 26 hrs. Dose fractionation studies (q24h, q12h and q6h) were performed at 5 ascending dose levels. Pharmacokinetic (PK) parameters (Cmax, AUC, T>MIC) in plasma and epithelial lining fluid (ELF) in infected animals were determined over the same dose range. The dose vs. change in log CFU/lung relationship was determined for each organism and related to the calculated PK parameters at each dose. Protein binding was determined by rapid equilibrium dialysis.

Results: TP-271 exhibited potent activity with static doses of ≤ 1 mg/kg against all three MRSA strains. PK analysis showed that free TP-271 levels were 2 – 5 fold higher in ELF as compared to plasma. Overall, the PK/PD parameter for both plasma and ELF that best predicted efficacy of TP-271 was the AUC/MIC (R2= 0.68 – 0.74) followed closely by Cmax/MIC (R2= 0.61 – 0.73). T>MIC was least likely to predict TP-271 efficacy (R2= 0.25 – 0.57). The 24 hour free AUC/MIC ratios necessary to achieve a static effect in the plasma and ELF ranged from 3.9 – 4.5 and 7.5 – 8.7, respectively. AUC/MIC ratios required to achieve 1 and 2-log10 CFU reductions were ~ 2 – 15x higher than those that resulted in a static effect for all organisms. Protein binding studies showed 2.7 – 26% free drug in plasma and 7.0 – 55% in ELF with greater % free drug at lower concentrations (100 – 100,000 ng/mL).

Conclusion: TP-271 efficacy was best predicted by the AUC/MIC parameter, which is consistent with other the tetracycline-class drugs.

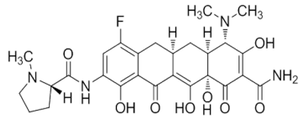
Introduction

TP-271 is a novel fluorocycline antibiotic currently in preclinical development for oral / IV treatment of complicated community acquired bacterial pneumonia (CABP) caused by susceptible and drug-resistant public health pathogens, pneumonic tularemia and other serious respiratory bacterial infections, including those caused by other biothreat pathogens. TP-271 has excellent potency against Gram-negative and Gram-positive pathogens associated with respiratory tract infections and remains active in strains expressing the most prevalent tetracycline-specific resistance mechanisms (efflux and ribosomal protection) as well as in isolates expressing the less prevalent inactivating enzyme, Tet(X). The current study was undertaken to determine the pharmacokinetic / pharmacodynamic parameter that best predicts efficacy in a pneumonia infection with MRSA.

Methods and Materials

Mice: Female 5 - 6 week old CD-1 mice (18-22 gm).
Neutropenia: Female CD-1 mice were rendered neutropenic by IP injection of Cytoxan (cyclophosphamide) 150 mg/kg (-4 days) and 100 mg/kg (-1 day) pre-infection.
Lung Infection: A fresh overnight culture of each MRSA strain in Trypticase soy Broth + 5% blood was sub-passed and incubated for 5 hours then diluted to approx. 2 x 10e8 CFU/mL and 0.05 mL instilled intranasally into the nares of the anesthetized mice.
MICs: MICs for TP-271 were determined by microbroth dilution in accordance with CLSI guidelines.
Pharmacokinetics : TP-271 was administered intravenously at 5 selected doses (0.25 – 10 mg/kg), with 8 time points and N=3 mice in order to determine pharmacokinetic parameters (Cmax, AUC, %T>MIC) and their relationship to administered dose. Pharmacokinetics were performed in neutropenic, thigh infected animals to best predict compound levels in the efficacy studies. Urea concentrations were determined on ELF samples using a blood urea nitrogen kit (QuantiChrom) and urea correction was included with the final PK analysis.
Dose Fractionation: TP-271 was administered intravenously at 5 different total daily doses (selected from the dose ranging studies and covering a range from maximal to the no-effect level). Each total dose was given at 3 different regimens; q24hr, q12hr and q6hr. Efficacy in the lung infection model was compared to calculated PK parameters from each of the dose fractionation regimens.
Protein Binding: was determined by rapid equilibrium dialysis (RED) from 100 – 100,000 ng/mL.

Chemical Structure TP-271

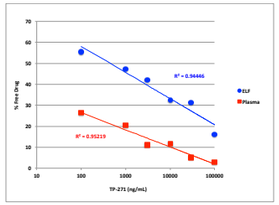


TP-271 Minimum Inhibitory Concentration (MIC)

UNT Strain Designation	Tetraphase Strain Designation	Phenotype	MIC (µg/mL)	MIC (ng/mL)
UNT138-3	SA191	Tet(M), ha-MRSA	0.0625	62.5
UNT141-3	SA527	TET-S, MACRO-R, FQ-R, MRSA	0.0625	62.5
UNT144-3	SA161	Tet(M), ha-MRSA	0.125	125

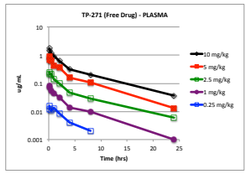
- TET-S, tetracycline-sensitive; MACRO-R, macrolide-resistant; FQ-R, fluoroquinolone-resistant; ha-MRSA, hospital-acquired MRSA. Drug-resistance phenotypes and genotypes were determined at Tetraphase Pharmaceuticals.
- MICs were previously determined at Tetraphase Pharmaceuticals according to CLSI methodology and the mode value of a total of 36 replicate assays, from 6 independent inocula.

Protein Binding Determination – TP-271

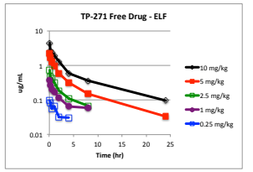


- Protein binding of TP-271 was higher in plasma than in ELF and the % free drug followed a linear relationship in both matrices.
- Increased protein binding was observed with increasing TP-271 concentrations which is comparable to that observed for Tigecycline and other tetracyclines.

Pharmacokinetics of TP-271 (Total Drug) Following Intravenous Administration to Female CD-1 Mice

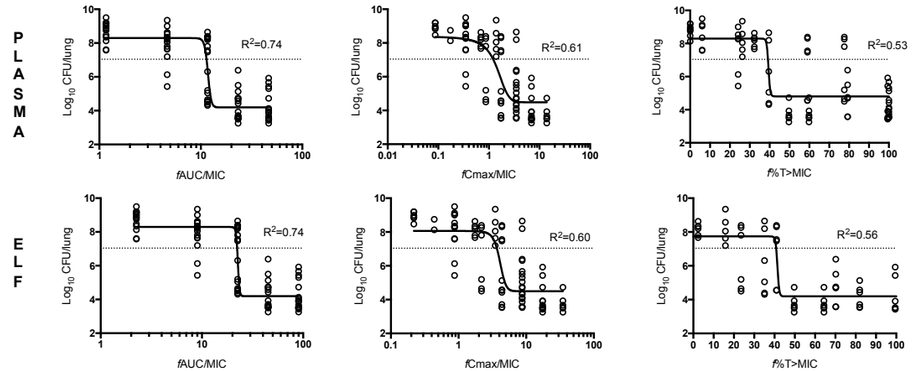


Parameter	10 mg/kg	5 mg/kg	2.5 mg/kg	1 mg/kg	0.25 mg/kg
C_{max} (µg/mL)	1.78	0.891	0.245	0.079	0.016
T_{max} (hr)	0.083	0.083	0.083	0.083	0.083
AUC $_{0-t}$ (µg-hr/mL)	5.96	2.98	0.894	0.277	0.048
AUC $_{0-inf}$ (µg-hr/mL)	6.23	3.08	0.945	0.284	0.055
Vd (L/kg)	13.24	11.66	22.71	23.97	17.28
Cl (L/hr/kg)	1.59	1.63	2.65	3.52	4.51
Half-life (hr)	5.7	4.9	5.9	4.7	2.7



Paramater	10 mg/kg	5 mg/kg	2.5 mg/kg	1 mg/kg	0.25 mg/kg
C_{max} (µg/mL)	4.43	2.25	0.728	0.372	0.097
T_{max} (hr)	0.083	0.083	0.083	0.083	0.083
AUC $_{0-t}$ (µg-hr/mL)	11.57	5.46	1.88	1.27	0.244
AUC $_{0-inf}$ (µg-hr/mL)	12.65	5.76	1.88	1.27	0.244
Vd (L/kg)	8.86	8.04	8.03	2.35	1.96
Cl (L/hr/kg)	0.791	0.878	1.33	0.786	1.022
Half-life (hr)	7.766	6.419	4.178	2.072	1.332

TP-271 (Free Drug) Efficacy / Pharmacodynamic Relationships in Plasma and ELF for *S. aureus* UNT144-3



Magnitude of Pharmacokinetic / Pharmacodynamic Parameters for TP-271 Efficacy in lung infection model with MRSA

		AUC/MIC						AUC (µg-hr/mL)					
		Plasma			ELF			Plasma			ELF		
Strain	TP-271	Static	1-log	2-log	Static	1-log	2-log	Static	1-log	2-log	Static	1-log	2-log
UNT138-3	Total Drug	32.83	243.8	454.77	20.65	153.27	285.91	2.05	15.24	28.42	1.29	9.58	17.87
	Free Drug	4.49	33.38	62.27	8.7	64.62	120.53	0.28	2.09	3.89	0.54	4.04	7.53
UNT141-3	Total Drug	28.1	85.1	246.57	17.3	53.5	155.03	1.76	5.32	15.41	1.08	3.34	9.69
	Free Drug	3.9	11.65	33.76	7.5	22.56	65.35	0.24	0.73	2.11	0.47	1.41	4.08
UNT144-3	Total Drug	28.65	105.74	182.84	18.02	66.48	114.95	3.58	13.22	22.86	2.25	8.31	14.37
	Free Drug	3.92	14.47	25.03	7.59	28.03	48.46	0.49	1.81	3.13	0.95	3.5	6.06

Correlation coefficients for PK/PD Curves

		R^2 (Correlation)			
		Total TP-271		Free TP-271	
Strain	Parameter	Plasma	ELF	Plasma	ELF
UNT138-3	AUC/MIC	0.69	0.68	0.69	0.69
	C_{max}/MIC	0.72	0.71	0.72	0.72
	%T>MIC	0.41	0.43	0.51	0.45
UNT141-3	AUC/MIC	0.66	0.66	0.66	0.66
	C_{max}/MIC	0.68	0.73	0.72	0.72
	%T>MIC	0.25	0.46	0.38	0.29
UNT144-3	AUC/MIC	0.74	0.74	0.74	0.74
	C_{max}/MIC	0.61	0.61	0.61	0.6
	%T>MIC	0.57	0.57	0.53	0.56

Summary and Conclusions

- TP-271 was active against the methicillin-resistant MRSA clinical isolates used in this study (MIC=0.0625 – 0.125 µg/mL).
- TP-271 exhibits dose-proportional pharmacokinetics following intravenous administration with excellent correlations for AUC and Cmax to dose.
- Free TP-271 concentration levels were 2 – 5 fold higher in ELF as compared to plasma.
- Protein binding of TP-271 was higher in plasma than in ELF and increased % binding was observed with increasing TP-271 concentrations (comparable to that observed for Tigecycline and other tetracyclines).
- The correlation coefficients of the PD parameters to efficacy in the lung model for the 24 hr AUC/MIC, Cmax/MIC and %T>MIC were 0.66 – 0.74, 0.61 – 0.72. and 0.25 – 0.57, respectively.
- Correlation coefficients for AUC/MIC and Cmax/MIC were comparable for strains UNT138-3 and 141-3, but the AUC/MIC was clearly more predictive for UNT144-3 (2-fold higher MIC than the other two strains).
- The 24 hr free AUC necessary to achieve a static effect in lung CFU for all three organisms were 0.24 – 0.49 µg-hr/mL and 0.47 – 0.95 µg-hr/mL in plasma and lung tissue, respectively.

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