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Pharmacodynamic Evaluation of TP-271, a novel Fluorocycline, in a Neutropenic Murine Lung Model Infected with *Streptococcus pneumoniae*

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Abstract

Background: TP-271 is a novel fluorocycline that is currently in preclinical development for the treatment of respiratory infections caused by susceptible and resistant pathogens including *S. pneumoniae*. We utilized a neutropenic mouse lung infection model to assess the pharmacokinetics (PK) and efficacy of TP-271 against 3 *S. pneumoniae* clinical isolates.

Methods: For all studies female CD-1 mice were rendered neutropenic through two intraperitoneal doses of cyclophosphamide, 150 and 100 mg/kg on Day -4 and Day -1, respectively. Mice were infected via intranasal inoculation under anesthesia with 10^6 colony forming units (CFUs) in 0.05 mL. TP-271 was administered at 12 hours post-infection. PK was performed in infected mice with plasma and epithelial lining fluid (ELF) collected following single IV doses ranging from 0.25 – 20 mg/kg. A urea correction was performed on the ELF samples. Dose fractionation studies were performed with dosing intervals at q24h, q12h and q6h. CFUs were determined in lung at the beginning of therapy and 24 hours later. PK parameters were determined using PK Solutions™ and PK-PD analysis was performed using GraphPad Prism. Protein binding was determined by rapid equilibrium dialysis.

Results: The following table summarizes the results:

S. pneumoniae strain:	UNT039-2	UNT040-2	UNT043-2
Phenotype:	Macrolide-R, Fluoroquinolone-R, Tetracycline-S	Tetracycline-S	Penicillin-R, Macrolide-R, Tet (M), Tet (O)
TP-271 MIC (µg/mL)	0.001	0.002	0.001
*f*AUC (µg-hr/mL) for Stasis in plasma / ELF	1.4 / 3.7	5.0/13.0	0.4 / 1.1
fAUC/MIC$_{plasma}$ (R^2)	0.69	0.62	0.73
fAUC/MIC$_{ELF}$ (R^2)	0.69	0.76	0.73
fCmax/MIC $_{plasma}$ (R^2)	0.74	0.49	0.64
fCmax/MIC $_{ELF}$ (R^2)	0.74	0.58	0.64

The *f* %T>MIC parameter for all 3 strains demonstrated R^2 values ≤ 0.5 for both plasma and ELF. The *f*AUC for TP-271 required to achieve a static effect in both plasma and ELF was 0.4 – 13.0 µg-hr/mL. Protein binding of TP-271 was higher in plasma than in ELF with a greater % free drug at lower TP-271 concentrations.

Conclusions: TP-271 demonstrated potent efficacy in the murine pneumonia model against all three *S. pneumoniae* tested. For two strains (UNT040-2 and 043-2) the *f*AUC/MIC for plasma and ELF was the predominant PK/PD indicator of efficacy. For UNT039-2, the *f*AUC/MIC and *f*Cmax/MIC were comparable. These results should enable dose selections of TP-271 in future clinical studies for bacterial pneumonia.

Introduction

TP-271 is a novel fully synthetic fluorocycline antibiotic that is currently in preclinical development for oral / IV therapy against complicated community-acquired bacterial pneumonia. TP-271 has demonstrated excellent activity against both Gram positive and Gram negative isolates associated with respiratory tract infections. TP-271 maintains antimicrobial activity in strains that express the most common tetracycline-specific resistance mechanisms (efflux and ribosomal) as well as Tet(X) inactivating enzyme. The purpose of these PK/PD studies is to elucidate which parameter best describes the pharmacodynamics of TP-271 to aid in dose selection and regimen for clinical development.

Methods and Materials

***In vitro* MICs:** Minimum inhibitory concentrations of all isolates were performed according to CLSI standards using broth micro-dilution method.

Animals: CD-1 female mice (weighing 18 – 22 grams) from Harlan (Indianapolis, IN) were acclimated for 48 hours prior to start of study. All studies were performed under approved IACUC protocols. Animals had free access to food and water throughout the study.

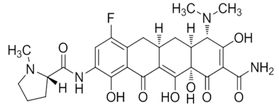
Neutropenia induction: mice were rendered neutropenic with two consecutive IP doses of cyclophosphamide (Cytoxan) of 150 and 100 mg/kg on 4 and 1 days prior to infection, respectively.

Inoculum preparation and infection: Infection inoculums were prepared from over night growth plates of *S. pneumoniae* strains. The isolates were resuspended in sterile saline and a predetermined density and further diluted in saline to achieve the infecting inoculum. Mice were anesthetized with an IP injection of Ketamine and xylazine. The prepared bacterial suspension was delivered in a 50 µL aliquot intranasally and the mice were returned to their cages to recover from the anesthesia.

Pharmacokinetics: TP-271 was delivered as a single intravenous injection over 5 selected dose concentrations. At 8 predetermined time points, 5 mice per time point were euthanized via CO_2 inhalation with blood collected for plasma and BAL (bronchial alveolar lavage) performed to collect ELF (epithelial lining fluid). Both plasma and ELF were analyzed for drug level concentrations. PK parameters were determined using PK Solutions™ software. Urea concentrations were determined on ELF samples using a blood urea nitrogen kit (QuantiChrom) and urea correction was included with the final PK analysis.

Dosing studies: Mice received a single dose of TP-271 over 5 selected concentrations via intravenous injection beginning at 12 hours post infection. Twenty-four hours after the start of dosing, mice were euthanized via CO_2 inhalation with lung aseptically removed. CFUs were determined after homogenizing, serial dilution, plating on bacterial growth media and over night incubation. An additional group of lungs were collected at start of treatment to serve as start of therapy controls. The total dose was also split and delivered both as a q12 hour and q6 hour dose regimen in order to elucidate the PD parameters. GraphPad Prism software was employed to determine which PK/PD parameter fits best in both plasma and ELF.

Chemical Structure TP-271



TP-271 Minimum Inhibitory Concentration (MIC)

UNT Strain Designation	Tetraphase Strain Designation	Phenotype[1]	MIC (µg/mL)	MIC (ng/mL)
UNT039-2	SP1517	MACRO-R, FQ-R, TET-S	0.001	1
UNT040-2	SP514	TET-S	0.002	2
UNT043-2	SP1579	PEN-R, MACRO-R, Tet(M), Tet(O)	0.001	1

[1]TET-S, tetracycline-sensitive; PEN-R, penicillin-R; MACRO-R, macrolide-resistant; FQ-R, fluoroquinolone-resistant

Pharmacokinetics of TP-271 Following Intravenous Administration to Female CD-1 Mice

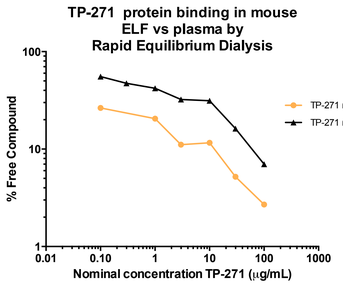
Single dose PK for free TP-271 in Plasma

Parameter	20 mg/kg	10 mg/kg	5 mg/kg	1 mg/kg	0.25 mg/kg
Cmax (µg/mL)	2.205	1.722	0.716	0.107	0.018
Tmax (hr)	0.083	0.083	0.083	0.083	0.083
AUC 0-t (µg-hr/mL)	11.416	5.858	2.739	0.288	0.045
Half-life (hr)	5.1	5.7	5	5.5	2.9

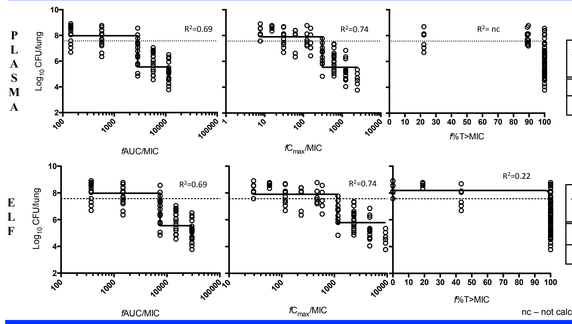
Single dose PK for free TP-271 in ELF

Parameter	20 mg/kg	10 mg/kg	5 mg/kg	1 mg/kg	0.25 mg/kg
Cmax (µg/mL)	9.948	3.752	1.835	0.479	0.133
Tmax (hr)	0.083	0.083	0.083	0.083	0.083
AUC 0-t (µg-hr/mL)	30.539	13.930	5.849	0.949	0.129
Half-life (hr)	5.419	5.946	5.18	3.338	6.544

Protein Binding Determination – TP-271



Dose Fractionation Murine Lung Infection – TP-271 vs. *S. pneumoniae* UNT039-2

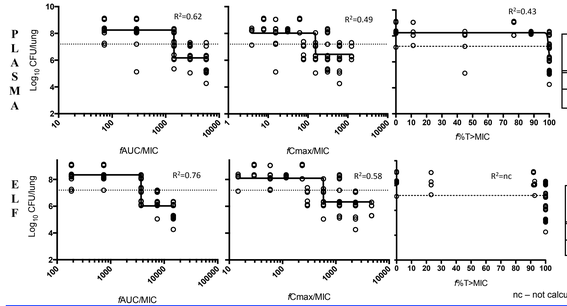


	PLASMA					
		AUC / MIC		AUC (µg*hr/mL)		
TP-271	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction
Free	1413	4230	7047	1.4	4.2	7.0
Total	15108	45195	75282	15.1	45.2	75.3

	ELF					
		AUC / MIC		AUC (µg*hr/mL)		
TP-271	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction
Free	3668	10972	18277	3.7	11.0	18.3
Total	10181	30456	50731	10.2	30.5	50.7

nc – not calculated

Dose Fractionation Murine Lung Infection – TP-271 vs. *S. pneumoniae* UNT040-2

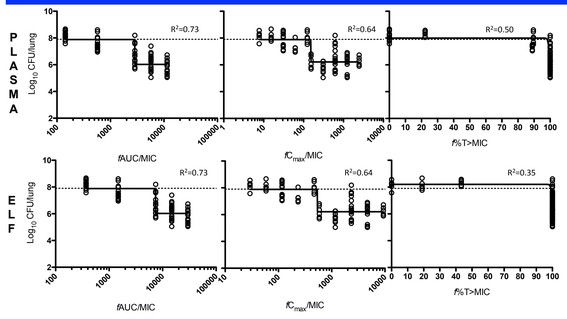


	PLASMA					
		AUC / MIC		AUC (µg*hr/mL)		
TP-271	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction
Free	2504	4501	6499	5.0	9.0	13.0
Total	26755	48092	69430	53.5	96.2	138.9

	ELF					
		AUC / MIC		AUC (µg*hr/mL)		
TP-271	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction
Free	6495	11676	16856	13.0	23.4	33.7
Total	18029	32408	46787	36.1	64.8	93.6

nc – not calculated

Dose Fractionation Murine Lung Infection – TP-271 vs. *S. pneumoniae* UNT043-2



	PLASMA					
		AUC / MIC		AUC (µg*hr/mL)		
TP-271	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction
Free	441	5858	11275	0.4	5.9	11.3
Total	4714	62577	120440	4.7	62.6	120.4

	ELF					
		AUC / MIC		AUC (µg*hr/mL)		
TP-271	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction	Static	1 $\log_{10}$ CFU reduction	2 $\log_{10}$ CFU reduction
Free	1144	15193	29241	1.1	15.2	29.2
Total	3177	42169	81162	3.2	42.2	81.2

Summary and Conclusions

- TP-271 demonstrated significant *in vitro* activity against both susceptible and resistant clinical isolates used in this study.
- TP-271 exhibited a dose-proportional pharmacokinetic profile in infected animals with significant accumulation at the site of infection (ELF).
- The protein binding of TP-271 was higher in plasma then ELF with a greater percentage of free drug at the lower drug concentrations.
- AUC/ MIC was determined to be the best predictor of efficacy for two strains. Strain UNT040-2 demonstrated R^2 values of 0.62 and 0.76 in plasma and ELF, respectively. For the UNT043-2 strain, R^2 values of 0.73 for both plasma and ELF were calculated.
- Strain UNT039-2 demonstrated a slightly better correlation for C_{max}/MIC with R^2 values of 0.74 for both Plasma and ELF. The AUC/MIC correlation for this strain was an R^2 of 0.69 for both plasma and ELF.
- For all three strains, % T> MIC was the least predictive of efficacy.
- The static AUC/MIC ratios for free drug ranged from 441 to 2504 in plasma and 1144 to 6495 in ELF.
- These data will assist in the dose selections for TP-271 in clinical studies of bacterial pneumonia.

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