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The Novel Fluorocycline TP-271 is Efficacious in a Murine *A. baumannii* Pneumonia Model

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Abstract

Background: TP-271 is a novel, fully-synthetic, broad-spectrum fluorocycline antibiotic that has entered phase 1 studies and is in clinical development for the treatment of respiratory infections caused by susceptible and resistant pathogens. TP-271 has demonstrated potent activity against both Gram-positive and Gram-negative isolates, including community-acquired respiratory, and problematic multidrug-resistant (MDR) pathogens. The purpose of these pharmacokinetic/ pharmacodynamic (PK/PD) studies was to evaluate the efficacy of TP-271 against four clinical carbapenem-resistant *A. baumannii* (CRAB) isolates in a neutropenic murine pneumonia model.

Methods: Female CD-1 mice were rendered neutropenic by IP injection of cyclophosphamide at 150 mg/kg (Day -4) and 100 mg/kg (Day -1) pre-infection. Mice were infected intranasally with approximately 7 $\log_{10}$ CFU. Intravenous (IV) TP-271 administration was initiated at 2hr post-infection and was administered QD (q24h), BID (q12h) and QID (q6h) in each dose fractionation study. Groups of infected mice were used for determining free plasma and epithelial lining fluid (ELF) concentrations. The exposure for each dose group was determined using the mean values for each time point in a 2-compartment model. The delta in CFU/lung was determined for each animal in the efficacy group and used in the PK/PD analysis.

Results: TP-271 free plasma and ELF exposures were non-linear across the IV dosing range employed, with TP-271 penetrating significantly into ELF. TP-271 demonstrated significant efficacy against all four CRAB isolates. Based upon ELF levels, fAUC/MIC was the PK/PD index best-associated with efficacy. TP-271 fAUC/MIC ratios associated with a 1-log to 2-log kill were 114 ± 16 and 153 ± 38, respectively.

Conclusions: TP-271 is highly effective against clinical *A. baumannii* strains *in vivo*. These results support the further evaluation of TP-271 to treat respiratory tract infections, including those with *A. baumannii*.

Background

TP-271 is a novel, fully-synthetic fluorocycline antibiotic in clinical development for oral / IV treatment of infections including complicated community acquired bacterial pneumonia (CABP) caused by susceptible and drug-resistant public health pathogens, pneumonic tularemia and other serious respiratory bacterial/biothreat infections. TP-271 has demonstrated potent *in vitro* activity against Gram-negative and Gram-positive pathogens associated with respiratory tract infections, including multidrug-resistant (MDR) organisms. Additionally, it 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). TP-271 is currently in phase 1 clinical trials.

The purpose of these pharmacokinetic/ pharmacodynamic (PK/PD) studies was to evaluate the efficacy of TP-271 against four clinical carbapenem-resistant *A. baumannii* (CRAB) isolates in a neutropenic murine pneumonia model.

Methods

Strain source

Four *A. baumannii* isolates were utilized in dose fractionation studies to assess the fAUC/MIC magnitudes associated with efficacy (Table1).

Table 1. *A. baumannii* isolates utilized in this study.

Isolate	Phenotype/genotype	TP-271 MIC (µg/mL)
UNT091-1	TEM, ADC-26, OXA	0.25
UNT235-1	tet(A), ADC-11, OXA	0.5
UNT236-1	OXA	0.0625
UNT237-1	tet(B), ADC-26, OXA	0.25

Free plasma and free ELF assessments:

The *in vitro* binding of TP-271 to pooled (n≥3) plasma and extracellular lining fluid (ELF) proteins was assessed by a rapid equilibrium dialysis (RED) method at concentrations ranging from 0.1 to 100 µg/mL.

Efficacy Experimental Design

Female CD-1 mice (22 ± 2 g) were rendered neutropenic by IP injection of cyclophosphamide (Cytoxan) at 150 mg/kg (Day -4) and 100 mg/kg (Day -1) pre-infection. Anesthetized mice were infected intranasally with 50 µL containing ~7 $\log_{10}$ CFU at time = 0. All four *A. baumannii* isolates established a stable infection, growing between 2-3 $\log_{10}$ CFU within 24 hours.

TP-271 was administered QD (q24h), BID (q12h) and QID (q6h) intravenously (IV) starting at 2hr post-infection at total daily doses of 10, 20, 40, 60, and 80 mg/kg/day in the efficacy studies.

To assess the pharmacokinetics of TP-271, it was administered at 1, 5, 10, 15, 20, 30, 40 and 60 mg/kg intravenously (IV) to mice that were intranasally inoculated with *A. baumannii* UNT091-1. Blood and bronchoalveolar lavage (BALF) samples were taken at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24 hr. The plasma and the urea corrected extracellular lining fluid (ELF) profile for each dose group was obtained by calculating the average concentration of TP-271 in each of the five animals per time point following a single IV administration. Free concentrations were calculated using the free plasma and ELF relationships. The PK properties for each dose group were determined by 2-compartment analysis (WinNonLin 5.2, Pharsight).

The correlation between efficacy and the PK/PD indices total AUC/MIC, C_{max}/MIC and %T>MIC were determined by non linear regression (WinNonlin 5.2, Pharsight). The data were modelled using a sigmoidal Emax Error! Reference source not found. shown below, where E_{max} is the maximum growth observed in the absence of drug; E0 is the maximum kill, EC_{50} is the concentration that gives 50% of response, and N is the Hill factor.

$$E = E_{max} - (E_{max} - E_0) \cdot \frac{C^N}{C^N + EC_{50}{}^N}$$

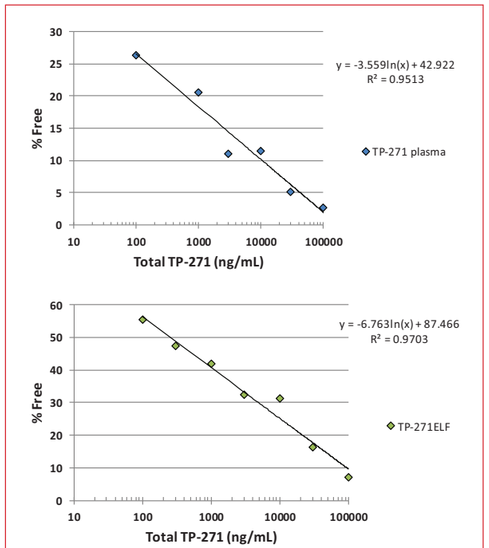
The PK/PD analysis was performed using the individual responses. The goodness of fit was determined by evaluating R^2 value for each PK/PD indices.

Results

TP-271 protein and ELF binding assessments:

- TP-271 protein binding is linear in both plasma and ELF. Like other tetracyclines, TP-271 protein binding increased as total drug concentration increased (Figure 1).
- In mouse plasma, the unbound fraction (% free) ranged from 26% free at 0.1 µg/mL to 2.7 % free at 100 µg/mL.
- TP-271 was not bound as extensively to mouse ELF, ranged from 56% free at 0.1 µg/mL to 7 % free at 100 µg/mL.

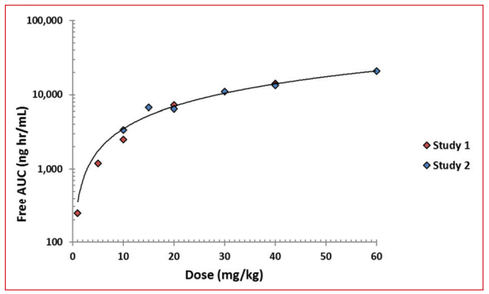
Figure 1. Percent of free TP-271 in mouse plasma and ELF.



ELF exposures in mice:

- Free ELF and free plasma concentrations were calculated for TP-271 following a single IV administration.
- Free ELF exposures did not differ significantly across two experiments (Figure 2).
- Free ELF exposures were approximately 80% of the free plasma concentrations across the dose range tested (1-60 mg/kg).
- The free ELF exposures were utilized for the PK/PD indices and magnitude determinations.

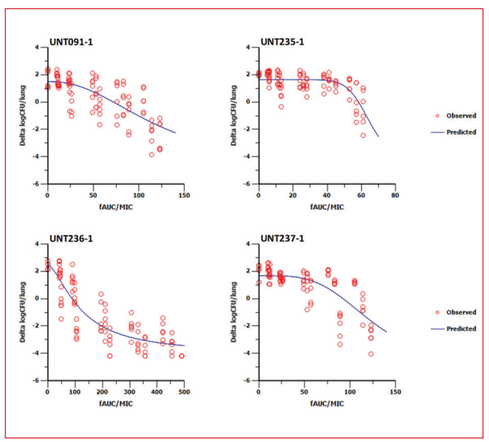
Figure 2. The free AUC ELF concentrations of TP-271 in mice following a single IV administration



A. baumannii efficacy studies:

- Based upon MIC-corrected exposures, similar results were seen for isolates with TP-271 MICs in the range 0.0625-0.5 µg/mL (Figure 3).
- An incomplete response was seen with UNT235 (TP-271 MIC = 0.5 µg/mL). The top dose assessed returned a fAUC/MIC exposure of ~60, this magnitude was associated with a static response for the other three isolates.

Figure 3. TP-271 efficacy results against four carbapenem-resistant *A. baumannii* clinical isolates.



TP-271 PK/PD assessments:

- For all three isolates the PK/PD indices best-associated with efficacy was fAUC/MIC (Table 2).
- The fAUC/MIC magnitudes required for stasis and 1- and 2-log reductions were generated for each individual isolate using a sigmoid Emax model (Table 3).

Table 2. PK/PD parameter fits associated free ELF levels of TP-271 in the *A. baumannii* mouse lung model.

Isolate	fAUC/MIC		fC_{max}/MIC		%T>fMIC	
	R^2	WSSR	R^2	WSSR	R^2	WSSR
UNT091-1	0.74	99	0.70	110	0.66	122
UNT236-1	0.86	116	0.83	142	0.82	151
UNT237-1	0.74	94	0.64	122	0.63	123

Table 3. TP-271 fAUC/MIC magnitudes associated with efficacy. Magnitudes are presented as Mean ± standard error of the mean (SEM).

Isolate	Stasis	1-log reduction	2-log reduction
	Mean ± SEM	Mean ± SEM	Mean ± SEM
UNT091-1	72 ± 6	100 ± 6	131 ± 18
UNT236-1	88 ± 8	131 ± 11	197 ± 19
INT237-1	92 ± 5	111 ± 5	131 ± 14
Mean ± SEM	84 ± 11	114 ± 6	153 ± 38

Conclusions

- TP-271 showed dose–dependent efficacy *in vivo* against four carbapenem-resistant *A. baumannii* in neutropenic mice.
- The PK/PD parameter best-associated with efficacy was the free ELF AUC/MIC.
- The mean free ELF AUC/MIC associated with a 1-log to 2-log kill were 114 ± 16 and 153 ± 38, respectively.
- These results support the further evaluation of TP-271 to treat respiratory tract infections, including those with *A. baumannii*.