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Pharmacokinetics-Pharmacodynamics of Lefamulin in a Neutropenic Murine Lung Infection Model

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ABSTRACT

Background: Lefamulin is an intravenous (IV) and oral semi-synthetic pleuromutilin antibiotic entering Phase 3 clinical development for community-acquired bacterial pneumonia (CABP). Lefamulin is active against pathogens commonly associated with CABP, including multi-drug resistant *S. pneumoniae*, *S. aureus,* and *M. pneumoniae*. *In vivo* studies were undertaken to determine lefamulin exposures associated with efficacy.

Methods: BALB/c mice ($n = 6$♀/group) were rendered neutropenic by 2 i.p. cyclophosphamide injections and inoculated (~6-$\log_{10}$ CFU/animal) with 1 of 10 challenge isolates. The challenge panel consisted of 5 *S. pneumoniae* and 5 *S. aureus* isolates. Two hours after inoculation, lefamulin treatment was initiated with doses ranging from 1.25 to 320 mg/kg/day given s.c. in 2 equally divided doses. Lefamulin concentrations were measured in plasma and epithelial lining fluid (ELF). After 24 h of treatment, mice were euthanized and lung tissue was harvested, homogenized, serially diluted, and plated for CFU determination. Hill-type models were used to describe relationships between change in $\log_{10}$ CFU at 24 h and lefamulin AUC:MIC ratio.

Results: MICs for *S. pneumoniae* and *S. aureus* were 0.12-0.5 µg/mL and 0.06-0.5 µg/mL, respectively. Relationships between AUC:MIC ratio and change in $\log_{10}$ CFU were well described by Hill-models (**Figure 1**: *S. pneumoniae*, r^2=0.65; *S. aureus*, $r^2 = 0.69$). Median plasma/ELF AUC:MIC ratios associated with a 1- and 2-$\log_{10}$ CFU reduction were 1.37/14.0 and 2.15/22.0 for *S. pneumoniae* and 2.13/21.7 and 6.24/63.9 for *S. aureus*, respectively.

Conclusions: These data are useful to support the selection of lefamulin 150 mg IV/600 mg oral Q12h for evaluation in future CABP studies.

INTRODUCTION

- Lefamulin is an antimicrobial agent from the pleuromutilin class that demonstrates *in vitro* microbiological activity against a wide range of bacterial pathogens, including common pathogens causing community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infection (ABSSSI) such as *Streptococcus pneumoniae* and *Staphylococcus aureus*, including methicillin-resistant isolates (MRSA).
- Lefamulin is being developed for intravenous (IV) and oral administration, thus allowing for a potential IV to oral switch (i.e., step-down therapy) in patients with CABP, ABSSSI, and other diseases.
- To support dose selection, the non-clinical pharmacokinetic-pharmacodynamic (PK-PD) targets for efficacy of lefamulin were determined using data from a neutropenic murine lung infection model caused by *S. pneumoniae* and *S. aureus*.
- Since previous dose-fractionation studies conducted using a neutropenic murine-thigh infection model have shown the ratio of the area under the concentration-time curve to minimum inhibitory concentration (AUC:MIC ratio) to be most predictive of lefamulin efficacy (Craig WA *et.al*, ICAAC 2010, Abstract F1-2108), this PK-PD index was the focus of the analyses described herein.

METHODS

Animals

- Female BALB/c mice weighing 20-30 grams were studied.

Challenge Organisms and Susceptibility Studies

- The challenge panel consisted of 5 *S. pneumoniae* and 5 *S. aureus* isolates, each of which was selected based on upon their virulence and their susceptibility. Efforts were made to include bacterial strains isolated from CABP patients (SENTRY 2010) with clinical relevant lefamulin MIC values including and exceeding the MIC_{90} values for *S. pneumoniae* (0.25 µg/mL) and *S. aureus* (0.12 µg/mL).
- Minimum inhibitory concentrations (MIC) were determined by broth microdilution methods according to CLSI (M07-A9, 2009).

Pharmacokinetics

- To determine pharmacokinetic parameters, 35 and 70 mg/kg of lefamulin were administered s.c. to non-neutropenic mice as a single dose.
- At seven time points between pre-dose and 5.5 h post-administration, plasma and bronchoalveolar lavage (BAL) samples were collected.
- All PK samples were analyzed by LC-MS/MS for lefamulin.
- The concentration of lefamulin in the epithelial lining fluid (ELF) within the BAL was determined by using the ratio of urea concentration found to that observed in plasma samples, collected simultaneously (BioAssay QuantiChrom™).
- The free-fraction of lefamulin in mouse plasma of 20 % was determined in an *in vitro* study using equilibrium dialysis.

Neutropenic Murine Lung infection Model and Dose-Ranging Studies

- Prior to the infection, mice were rendered neutropenic by cyclophosphamide given i.p. 4 days (at 150 mg/kg) and 1 day (at 100 mg/kg).
- Lung infection was induced by the intranasal inoculation of about 10^6 CFU of bacteria in 50 µL (adapted from Tateda K *et. al*; AAC 1996).
- Treatment was initiated 2 h post-inoculation with subcutaneous (s.c.) administered lefamulin doses of 1.25 to 320 mg/kg/day (group size $n = 6$) divided in two equally doses.
- At the end of the treatment period (24 h after start of therapy), the lungs of mice were aseptically removed and the number of viable organisms at the infection site was determined by agar plating.

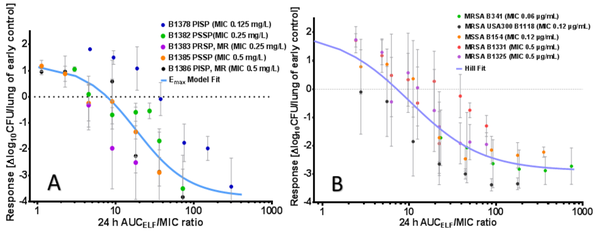
Data Analyses

- Relationships between response (ΔCFU/lung) following 24 h of treatment at exposure levels (plasma and ELF) compared to baseline and free-drug plasma and ELF exposure were evaluated for each isolate using Hill-type models. Such relationships based on data pooled for *S. pneumoniae* and *S. aureus* were also evaluated.
- Using the above-described relationships, AUC:MIC ratio targets associated with a 1- and 2-$\log_{10}$ CFU reduction from baseline for each pathogen was determined.

RESULTS

- The distribution of lefamulin from plasma to ELF following a single dose was rapid. The penetration ratio in mice (AUC_{ELF}/total AUC_{plasma}) following s.c. dosing was approximately 2-fold.
- Relationships between change in $\log_{10}$ CFU from baseline and free-drug plasma or ELF AUC:MIC ratio for *S. pneumoniae* and *S. aureus* were well described by Hill-models. Relationships based on the data pooled for each pathogen and ELF exposures are shown in **Figure 1** (*S. pneumoniae*, $r^2 = 0.65$; *S. aureus*, $r^2 = 0.69$). Free-drug plasma and ELF AUC:MIC ratio targets associated with 1- and 2-$\log_{10}$ CFU reductions from baseline for *S. pneumoniae* and *S. aureus*, based on individual Hill-type models for each isolate, are shown in **Table 1** and **Table 2**, respectively.
  - Median plasma/ELF AUC:MIC ratios associated with a 1- and 2-$\log_{10}$ CFU reduction from baseline were 1.37/14.0 and 2.15/22.0 for *S. pneumoniae* (**Table 1**).
  - Median plasma/ELF AUC:MIC ratios associated with a 1- and 2-$\log_{10}$ CFU reduction from baseline were 2.13/21.7 and 6.24/63.9 for *S. aureus* (**Table 2**).
  - Given that the ELF AUC:MIC ratio targets reflect effect site exposures, emphasis was given to results based on this matrix.

Figure 1. Relationships between change in bacterial burden from baseline and lefamulin ELF AUC:MIC ratio based on data for *S. pneumoniae* (A) and *S. aureus* (B) isolates in a neutropenic murine lung infection model



PSSP, penicillin-susceptible *S. pneumoniae*
PISP, penicillin-intermediate *S. pneumoniae*
PRSP, penicillin-resistant *S. pneumoniae*
MR, macrolide resistant

MRSA, methicillin-resistant *S. aureus*
MSSA, methicillin-susceptible *S. aureus*

Table 1. Lefamulin AUC:MIC ratio targets for efficacy of *S. pneumoniae* isolates studied *in* a neutropenic murine lung infection model

Isolate ID	MIC [µg/mL]	1-$\log_{10}$ CFU reduction		2-$\log_{10}$ CFU reduction	
		Free-drug plasma AUC:MIC ratio	ELF AUC:MIC ratio	Free-drug plasma AUC:MIC ratio	ELF AUC:MIC ratio
B1378	0.12	6.05	61.8	10.7	109
B1382	0.25	2.73	27.9	3.96	40.5
B1383	0.25	0.67	6.84	1.06	10.8
B1385	0.5	1.34	13.7	2.15	22.0
B1386	0.5	1.37	14.0	1.66	17.0
Mean		2.43	24.9	3.91	39.9
Median		**1.37**	**14.0**	**2.15**	**22.0**
Min, Max		0.67, 6.05	6.84, 61.8	1.06, 10.7	10.8, 109

Table 2. Lefamulin AUC:MIC ratio targets for efficacy of *S. aureus* isolates studied in a neutropenic murine lung infection model

Isolate ID	MIC [µg/mL]	1-$\log_{10}$ CFU reduction		2-$\log_{10}$ CFU reduction	
		Free-drug plasma AUC:MIC ratio	ELF AUC:MIC ratio	Free-drug plasma AUC:MIC ratio	ELF AUC:MIC ratio
B154	0.12	1.69	17.2	3.42	34.9
B341	0.06	5.94	60.7	8.39	85.7
B1118	0.12	0.76	7.72	1.42	14.5
B1325	0.5	2.13	21.7	6.25	64.0
B1331	0.5	4.35	44.5	15.3	157
Mean		2.97	30.4	6.96	71.2
Median		**2.13**	**21.7**	**6.24**	**63.9**
Min, Max		0.76, 5.94	7.72, 60.7	1.42, 15.3	14.5, 157

CONCLUSIONS

- **Based on data from the neutropenic murine lung infection model, the pharmacodynamics of lefamulin showed good *in vitro* to *in vivo* correlation.**
- **Free-drug plasma and ELF AUC:MIC ratio targets for efficacy described herein will be useful to support dose selection of lefamulin for the treatment of patients with CABP and to identify pre-clinical susceptibility breakpoints for *S. pneumoniae* and *S. aureus*.**

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