Pharmacokinetic-Pharmacodynamic Characterization of Omadacycline Against *Haemophilus influenzae* Using a One-Compartment *In Vitro* Infection Model

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ABSTRACT

Introduction: Community-acquired bacterial pneumonia (CABP) is a major source of morbidity and mortality and is caused by a variety of bacteria including *Haemophilus influenzae*. Omadacycline is a novel aminomethylcycline with activity against Gram-positive and –negative organisms, including *H. influenzae*. The evaluation of *H. influenzae* using standard murine *in vivo* models is traditionally difficult due to low pathogenicity of this species in mice. Given these issues, a series of *in vitro* studies were undertaken with the goal of characterizing the magnitude AUC:MIC ratios associated with various bacterial reduction endpoints for a panel of *H. influenzae* clinical isolates.

Methods: A series of 24-hour dose-ranging studies were completed using a one-compartment *in vitro* infection model in which five clinical *H. influenzae* isolates (omadacycline MIC = 1 to 2 mg/L) were exposed to omadacycline concentration-time profiles based on those observed in human epithelial lining fluid (ELF) following intravenous administration. The relationship between change in $\log_{10}$ colony forming units (CFU) from baseline at 24 hours and total-drug ELF AUC:MIC ratio was evaluated and values associated with achieving net bacterial stasis and 1- and 2-$\log_{10}$ CFU reductions from baseline at 24 hours were determined.

Results: As evidenced by the high coefficient of determination (r^2) of 0.88 and the dispersion of data about the fitted line shown in Figure 3, total-drug ELF AUC:MIC ratio described the data well. The magnitude of total-drug ELF AUC:MIC ratio associated with net bacterial stasis and 1- and 2-$\log_{10}$ CFU/mL reductions from baseline at 24 hours was 5.87, 7.87, and 10.4, respectively.

Conclusions: Total-drug ELF AUC:MIC ratios associated with efficacy for *H. influenzae* were determined using an *in vitro* infection model. These data help to provide support for the omadacycline dosing regimens selected for patients with CABP, as well as susceptibility breakpoints for *H. influenzae*.

INTRODUCTION

- *Haemophilus influenzae* is an important cause of community-acquired bacterial pneumonia (CABP), especially in elderly patients and adults with chronic obstructive pulmonary disease [1].
- Omadacycline is a novel aminomethylcycline that has shown activity against Gram-positive and –negative organisms, including *H. influenzae* [2].
- Given the challenge of achieving sufficient growth control for *H. influenzae* using an *in vivo* infection model, *in vitro* infection models provide the opportunity to study this pathogen.
- Herein, we describe a series of one-compartment *in vitro* studies undertaken to determine the ratio of the total-drug epithelial lining fluid (ELF) area under the omadacycline concentration-time curve over 24 hours ($AUC_{0\text{-}24}$) to the minimum inhibitory concentration (MIC) ($AUC_{0\text{-}24}$:MIC ratio) associated with efficacy against a panel of clinical *H. influenzae* isolates.

OBJECTIVES

- The objectives of these studies were the following:
  - To evaluate the susceptibility profiles and frequency of mutation of clinical *H. influenzae* isolates to omadacycline; and
  - To determine the magnitude of the omadacycline total-drug ELF $AUC_{0\text{-}24}$:MIC ratio associated with efficacy, for a clinical panel of *H. influenzae* isolates evaluated using the one-compartment *in vitro* infection model.

METHODS

Antimicrobial Agents and Challenge Isolates

- Omadacycline was provided by Paratek Pharmaceuticals, Inc. (King of Prussia, PA).
- A panel of five clinical *H. influenzae* isolates was purchased from the American Type Culture Collection and JMI laboratories (North Liberty, IA).

METHODS

In Vitro Susceptibility Testing and Mutation Frequency Studies

- Omadacycline MIC values were determined in triplicate over a two-day period using Haemophilus test medium (HTM) microbroth- and agar-dilution methodologies, as per Clinical and Laboratory Standards Institute guidelines [3].
- The frequency of omadacycline resistance was estimated in duplicate for the five clinical *H. influenzae* isolates in the challenge panel by plating two milliliters of logarithm (log)- phase growth suspension onto HTM agar containing three- or five-times the omadacycline agar MIC.
- The ratio of isolates found on the drug-containing plates to that of the total population was used to estimate the frequency of resistance.

One-Compartment *In Vitro* Infection Model Dose-Ranging Studies

- A one-compartment *in vitro* infection model consisting of a central infection compartment containing fresh HTM broth, the challenge isolate, and a magnetic stir bar to ensure homogeneity, was placed in an incubator set at 35°C.
- Drug-free medium was pumped in and out of the central compartment via computer-controlled peristaltic pumps in order to simulate omadacycline total-drug ELF concentration-time profiles observed after intravenous (IV) administration every 12 hours (q12h) in healthy volunteers.
- Computer controlled syringe pumps were used to simulate selected beta and terminal half-lives of 1 hour and 14.9 hours, respectively.
- A suspension of each challenge isolate was prepared at a concentration of 1.0 x 10^6 colony forming units per milliliter (CFU/mL), and exposed to a range of 25 to 400 mg of omadacycline administered q12h.
- Samples were collected from the central infection compartment for CFU determination and drug concentration analysis at pre-determined time points throughout the duration of the study.
- Duplicate 24-hour studies were completed for each *H. influenzae* isolate.

Pharmacokinetic-Pharmacodynamic Analysis

- Pharmacokinetic-pharmacodynamic (PK-PD) data from the one-compartment *in vitro* models were evaluated using Hill-type models and non-linear squares regression.

RESULTS

In Vitro Susceptibility Testing

- The omadacycline MIC values determined for the panel of five clinical *H. influenzae* isolates are presented in **Table 1**. The omadacycline MIC values ranged from 1 to 2 mg/L, and 0.5 to 1 mg/L for the microbroth- and agar-dilution methods, respectively.

Table 1. Average frequency of omadacycline resistance for the five clinical *H. influenzae* isolates examined over two separate trials at 48 hours post inoculation[a]

H. influenzae isolate	Baseline omadacycline MIC (mg/L)		Mutation frequency inoculum (CFU/mL)	48-hour observation	
	Microbroth	Agar dilution		3x MIC	5x MIC
ATCC 49247	2	1	1.3×10^9	$<7.7 \times 10^{-10}$	$<7.7 \times 10^{-10}$
437	1	0.5	2.3×10^9	$<4.3 \times 10^{-10}$	$<4.3 \times 10^{-10}$
543	2	1	2.1×10^9	$<4.8 \times 10^{-10}$	$<4.8 \times 10^{-10}$
10929	1	1	1.7×10^9	$<5.9 \times 10^{-10}$	$<5.9 \times 10^{-10}$
2696	2	1	2.9×10^9	$<3.5 \times 10^{-10}$	$<3.5 \times 10^{-10}$

a. Results of omadacycline susceptibility testing and mutation frequency studies based on data for the five clinical *H. influenzae* isolates evaluated in the *in vitro* studies.

RESULTS

Mutation Frequency Studies

- As shown in **Table 1**, these mutation frequency studies failed to produce or identify a drug-resistant subpopulation for all five *H. influenzae* isolates evaluated. These data suggest that the frequency of mutations resulting in omadacycline resistance is less than or equal to the largest inocula examined of 3.44×10^{-10} CFU/mL for *H. influenzae*.

One-Compartment *In Vitro* Infection Model

- The targeted ELF concentration-time profiles for omadacycline were well simulated in the one-compartment *in vitro* infection model for all omadacycline dosing regimens, as evidenced by the agreement between the observed and predicted concentrations shown in **Figure 1** and **Figure 2**.
- The relationship between change in $\log_{10}$ CFU/mL from baseline at 24 hours and the omadacycline total-drug ELF $AUC_{0\text{-}24}$:MIC ratio was examined based on pooled data as shown in **Figure 3**.
  - As evidenced by the coefficient of determination (r^2) value of 0.88 and the dispersion of data about the fitted line, total-drug ELF $AUC_{0\text{-}24}$:MIC ratio described the efficacy of omadacycline well across the clinical *H. influenzae* isolates, with MIC values ranging from 1 to 2 mg/L.
- As described in **Table 2**, the magnitude of the total-drug ELF $AUC_{0\text{-}24}$:MIC associated with net bacterial stasis and 1- and 2-$\log_{10}$ CFU/mL reductions from baseline at 24 hours was 5.87, 7.87, and 10.4, respectively, based on the Hill model fit to the pooled data.

Figure 1. The relationship between predicted and observed omadacycline ELF concentrations simulated within the one-compartment *in vitro* infection models

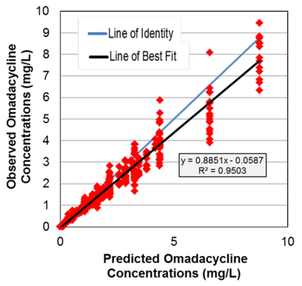

Figure 2. Predicted omadacycline ELF concentration-time profile with observed concentrations overlaid based on a omadacycline 100 mg 12h dosing regimen

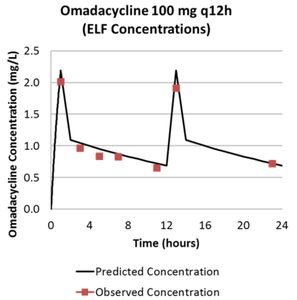

Figure 3. Relationship between the change in $\log_{10}$ CFU/mL from baseline at 24 hours and omadacycline total-drug ELF $AUC_{0\text{-}24}$:MIC ratio based on data for five clinical *H. influenzae* isolates examined in the dose-ranging studies

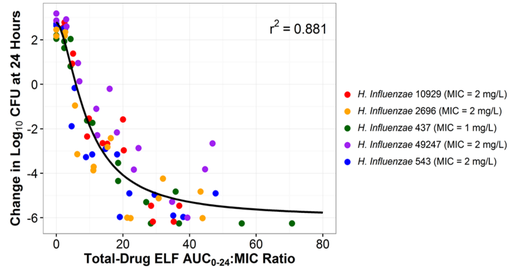

Table 2. Omadacycline total-drug ELF $AUC_{0\text{-}24}$:MIC ratio targets associated with various levels of bacterial reduction from baseline for *H. influenzae*

H. influenzae isolate	Total-drug ELF $AUC_{0\text{-}24}$:MIC ratio targets		
	Net bacterial stasis	1-$\log_{10}$ CFU reduction from baseline	2-$\log_{10}$ CFU reduction from baseline
ATCC 49247	8.76	11.6	15.5
437	6.91	8.91	11.1
543	4.45	5.78	7.45
10929	7.09	9.73	12.9
2696	4.38	5.44	6.72
Mean (SD)	6.32 (1.88)	8.30 (2.64)	10.7 (3.69)
Median	6.91	8.91	11.1
Pooled[a]	5.87	7.87	10.4

a. Based on the Hill-model fit of the data pooled across all isolates.

CONCLUSIONS

- Omadacycline MIC values for all *H. influenzae* isolates studied in the one-compartment *in vitro* infection model ranged from 1 to 2 mg/L.
- The frequency of mutation to omadacycline for the *H. influenzae* isolates evaluated could not be identified for bacterial populations less than or equal to 3.44×10^{-10} CFU/mL.
- As evidenced by a high r^2 of 0.88, the relationship between change in $\log_{10}$ CFU/mL from baseline at 24 hours and total-drug ELF $AUC_{0\text{-}24}$:MIC ratio described the efficacy of omadacycline against *H. influenzae* well.
  - The omadacycline total-drug ELF $AUC_{0\text{-}24}$:MIC ratio required to achieve net bacterial stasis, and 1- and 2-$\log_{10}$ CFU/mL reductions from baseline at 24 hours for *H. influenzae* was 5.87, 7.87, and 10.4, respectively. The median total-drug ELF $AUC_{0\text{-}24}$:MIC ratios associated with these endpoints for the isolates evaluated individually were 6.91, 8.91, and 11.1, respectively.

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