Evaluation of the *In Vitro* Activity Profile of Omadacycline Against *Haemophilus influenzae*

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ABSTRACT

Background: Omadacycline is a novel aminomethylcycline in Phase 3 clinical development for use against multiple bacterial pathogens, including *H. influenzae*. In order to evaluate the *in vitro* activity of omadacycline, a series of foundation studies were undertaken.

Methods: *In vitro* studies were performed using a panel of 5 *H. influenzae* clinical isolates. The studies included evaluating the MIC, the resistance mutation frequency, static time-kill activity and the post-antibiotic effect of omadacycline. All studies were performed using the Clinical Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing guidelines for broth and agar methodologies using their respective growth mediums. (Haemophilus Test Medium and Mueller Hinton broth medium supplemented with 5% defibrinated horse blood, plus 20 mg/L β-nicotinamide adenine dinucleotide MH-F).

Results: The MIC values for omadacycline ranged from 0.5 to 2 mg/L using broth and agar dilution methodologies. No isolates were recovered from the omadacycline drug-containing agar plates utilized in the mutation frequency studies at concentrations of 3 times the baseline MIC, using an average inoculum of 6.2 x 10^9 CFU/mL. These results demonstrated omadacycline's resistance mutation frequency to be below detection for the 5 clinical isolates. The results of the static time-kill studies showed that concentrations of 1 to 2 times the omadacycline MIC achieved a ≥3 Log_{10} CFU reduction from baseline. The median (min, max) duration of the PAE for omadacycline was 2.45 (1 to 4) hrs when examined against four clinical *H. influenzae* isolates from the original panel.

Conclusion: Bactericidal *in vitro* activity for *H. influenzae* was observed at omadacycline concentrations either matching or twice the MIC value for each individual isolate. This bactericidal activity along with low resistance mutation frequency demonstrate potent activity of omadacycline against *H. influenzae* and warrant further *H. influenzae* studies.

INTRODUCTION

- Omadacycline, a novel aminomethylcycline synthesized by the chemical modification of minocycline, has been shown to have activity against Gram-positive and-negative organisms. Such activity includes but is not limited to the following pathogens:
  - *Streptococcus pneumoniae*, methicillin resistant *Staphylococcus aureus, Enterococcus* species, and *Haemophilus influenzae* [1].
- Omadacycline is currently in Phase 3 clinical development for treatment of patients with acute bacterial skin and skin structure infections; as well as community-acquired bacterial pneumonia via both oral and intravenous (IV) administration [2].
- To date, little data has been generated describing the *in vitro* activity that omadacycline has against *H. influenzae*. Due to this lack of data, a series of *in vitro* studies were completed in order to evaluate the *in vitro* activity of this novel compound using various methodologies.

OBJECTIVES

- Using a panel of *H. influenzae* clinical isolates, the objectives of these analyses were the following:
  - To evaluate the *in vitro* activity of omadacyline using standard clinical laboratory methodologies utilized in hospitals in Europe and North America;
  - To assess the frequency of spontaneous omadacycline resistance using agar-based mutation frequency assays;
  - To evaluate the effect of omadacycline on populations of *H. influenzae* over the course of 24 hours using a series of static time-kill assays; and
  - To determine the post-antibiotic effect (PAE) omadacycline has upon *H. influenzae*.

METHODS

Test Compound

- Omadacycline was supplied by Paratek Pharmaceuticals, Inc. (Boston, MA).

Bacterial Isolates

- The reference strain utilized was obtained from American Type Culture Collection (ATCC) (Manassas, VA). Clinical isolates were obtained from JMI Laboratories (North Liberty, IA).

METHODS

Minimum Inhibitory Concentration Assays

- Omadacycline susceptibility studies were completed in triplicate over a two-day period, using freshly prepared medium, following both Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [3, 4].
- MIC values were determined using both micro broth and agar dilution assays, which have different requirements depending upon the methodology.
  - CLSI methodology required the use of *Haemophilus* Test Medium (HTM), 35°C + 5% CO_2 incubation for agar MIC values, and ambient air incubation for micro-dilution.
  - EUCAST methodology required the use of Mueller-Hinton medium supplemented with 5% mechanically defibrinated horse blood, and 20 mg/L β-nicotinamide adenine dinucleotide (MH-F), incubated at 35°C + 5% CO_2.

Mutation Frequency Studies

- The frequency of omadacycline resistance within a bacterial population was determined by plating 2 mL of log phase growth bacteria onto freshly prepared agar (HTM or MH-F), containing 3- or 5-times the corresponding agar MIC.
- The starting inoculum for each isolate was determined using drug-free chocolate agar plates (Becton Dickson, Franklin Lakes, New Jersey).
- The plates were incubated overnight per CLSI and EUCAST guidelines [3, 4].
- The ratio of the colony forming units (CFUs) found on the drug-containing plates to that of the starting inoculum provided an estimate of the rate of omadacycline mutation.

In Vitro Static Time-Kill Studies

- Time-kill studies were completed in duplicates using freshly prepared HTM or MH-F for five isolates chosen by their omadacycline MIC values.
- The bacterial starting inoculum was prepared from cultures grown overnight on chocolate agar and then suspended in each corresponding broth medium.
  - Broth suspensions were diluted to 1.0 x 10^6 CFU/mL using a previously determined growth curve. The suspension was made in 125 ml Erlenmeyer flasks, and incubated in a shaking water bath set to 35°C and 125 revolutions per minute.
- Omadacycline concentration ranging from treatment groups included a no-treatment control and serial two-fold concentrations of omadacycline ranging from 0.25- to 8-times the baseline HTM + 5% CO_2 broth MIC.
- Quantitative cultures were taken at the initiation of the study, and then at 2, 4, 8, 12, and 24 hours.
- Samples were washed twice in sterile normal saline to eliminate drug carryover, then serially diluted and plated on chocolate agar plates for enumeration of bacterial burden.

Post-Antibiotic Effect Studies

- PAE studies were completed in duplicates using freshly prepared HTM or MH-F for five isolates.
- The starting inoculum was prepared from cultures grown overnight on chocolate agar then suspended in the corresponding medium.
- Broth suspensions were diluted to 1.0 x 10^6 CFU/mL, placed in 125 mL Erlenmeyer flasks, and incubated at 35°C in a shaking water bath set to 125 revolutions per minute (rpm).
- Two conditions were evaluated per isolate, a no-treatment control, and 10 times the omadacycline broth MIC.
  - Both conditions were allowed to grow for one hour, after which the contents of the flasks were centrifuged at 1200 rpm, the supernatant was discarded, and the pellet was re-suspended in fresh media.
- The flasks are placed in the water bath, this is considered time zero, after which the suspensions are quantitatively cultured on chocolate agar every hour for up to ten hours.
- Quantitatively cultured plates are incubated overnight at 35°C + 5% CO_2. Viable counts are taken, and the two groups are compared to determine PAE.
- The PAE is calculated by subtracting the time (C), in hours, for the no-treatment control group to increase by 1 log_{10} CFU/ mL from the time for treatment group to increase 1 log_{10} CFU/mL from hour zero (T).

RESULTS

Minimal Inhibitory Concentration

- Omadacycline susceptibility test results are shown in **Table 1.** Omadacycline MIC values ranged from 0.25 to 2 mg/L for both methodologies.

Table 1. Modal values for omadacycline micro broth and agar dilution MIC values in HTM and MH-F medium

Isolate	Omadacyline MIC (mg/L) values			
	CLSI methods		EUCAST methods	
	Microbroth	Agar	Microbroth	Agar
H. influenzae ATCC 49247	2	1	2	0.5
H. influenzae 437	1	0.5	0.5	0.25
H. influenzae 543	2	1	1	0.5
H. influenzae 10929	2	1	1	0.5
H. influenzae 1253	2	2	2	2
H. influenzae 2696	2	1	2	0.5
H. influenzae 49766	1	0.25	0.5	0.25

Mutation Frequency

- No isolates were observed on any drug-containing plates for the inoculums and concentrations examined, regardless of growth medium utilized (**Table 2.**)

Table 2. Results of the omadacycline mutation frequency assays completed using growth mediums recommended in CLSI and EUCAST guidelines.

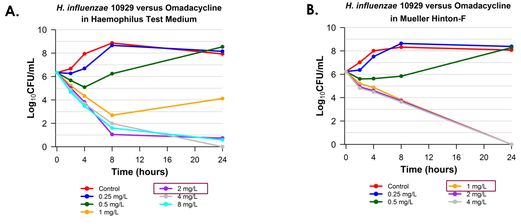
Isolate	Mutation frequency assay results at 48 hours							
	HTM agar (CLSI)				MH-F agar (EUCAST)			
	Baseline omadacycline agar MIC (mg/L)	Inoculum (CFU/mL)	MF @ 3 x MIC	MF @ 5 x MIC	Baseline omadacycline agar MIC (mg/L)	Inoculum (CFU/mL)	MF @ 3 x MIC	MF @ 5 x MIC
H. influenzae ATCC 49247	1	1.3 x 10^9	< -9.1	< -9.1	0.5	6.7 x 10^8	< -8.8	< -8.8
H. influenzae 437	0.5	2.3 x 10^9	< -9.4	< -9.4	0.25	4.4 x 10^8	< -8.6	< -8.6
H. influenzae 543	1	2.1 x 10^9	< -9.3	< -9.3	0.5	8.9 x 10^8	< -8.9	< -8.9
H. influenzae 10929	1	1.7 x 10^9	< -9.2	< -9.2	0.5	1.8 x 10^9	< -9.2	< -9.2
H. influenzae 1253	2	2.9 x 10^9	< -9.5	< -9.5	2	4.9 x 10^8	< -8.7	< -8.7
H. influenzae 2696	1	2.4 x 10^9	< -9.4	< -9.4	0.5	1.2 x 10^9	< -9.1	< -9.1

Note: Mutation frequencies are reported as < 1 resistant CFU in X Log_{10} C FU/mL. For example, *H. influenzae* 49247 on HTM has < 1 resistant CFU in every 9.1 Log_{10} CFU/ml of bacteria.

In Vitro Static Time-Kill Studies

- Omadacycline concentrations of 1 to 2 x the MIC produced a ≥ 3 log_{10} CFU/mL reduction from baseline over 24-hours for each of the five isolates. An example of omadcycline's bactericidal effect against one of the five *H. influenzae* clinical isolates is shown in **Figures 1 A and B.** The omadacycline MIC values for this isolate were 2 and 1 mg/L for HTM and MH-F, respectively.

Figure 1 A and B . Results of the *in vitro* omadacycline static time-kill study for omadacycline against *H. influenzae* 10929 using HTM (A) and MH-F (B)



Note: Red box denotes *H. influenzae* 10929 MIC for each broth medium, 2 mg/L in HTM and 1 mg/L in MH-F.

RESULTS

Post-Antibiotic Effect Studies

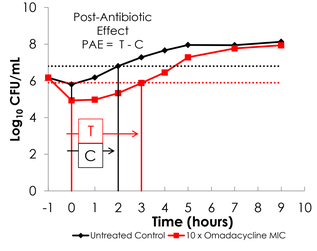
- The median (min, max) PAE was 2.45 (1.0, 4.0) hours (**Table 3**).
  - One isolate, *H. influenzae* 1253, grew poorly in HTM and the PAE could not be determined. The study was repeated using MH-F as a replacement.
- **Figure 3** shows the PAE of *H. influenzae* 1253 in MH-F. The PAE was determined to be 1 hour according to the calculation PAE = T-C.

Table 3. PAE of omadacycline for a panel of four *H. influenzae* isolates in HTM

Isolate	Concentration of omadacycline utilized in 10 x MIC drug containing regimen (mg/L)	Post-antibiotic effect (hours)
H. influenzae 437	5	4.01
H. influenzae 543	10	1.00
H. influenzae 10929	10	3.90
H. influenzae 1253	20[a]	1.00[a]

a. Performed in MH-F broth, due to poor growth in HTM

Figure 3. The PAE of *H. influenzae* 1253 in MH-F



CONCLUSIONS

- The results of these *in vitro* studies to support future investigations of omadacycline activity against *H. influenzae* as evidenced by the following:
  - Relatively low MIC values ranging from 0.25 to 2 mg/L for both CLSI and EUCAST methodologies;
  - A frequency of omadacycline resistance which was lower than the inoculums and concentrations examined for both methodologies;
  - The time-kill studies providing bactericidal concentrations of omadacycline similar to the MIC values determined in a static environment over 24 hours; and
  - A median PAE of 2.45 hours after being removed from the presence of *H. influenzae*.

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Acknowledgements

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