Activity and Efficacy of Omadacycline against *Clostridium difficile*

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ABSTRACT

Introduction: Omadacycline (OMC) is a new aminomethylcycline in Phase 3 clinical development for the oral and intravenous treatment of acute bacterial skin and skin structure infections (ABSSSI) and community acquired bacterial pneumonia (CABP). OMC has broad-spectrum activity against Gram-positive, Gram-negative, and atypical bacteria.

Material and methods: OMC was tested *in vitro* against 27 clinical isolates of *C. difficile* (Cd) using both broth microdilution and agar dilution methods according to CLSI guidelines. The efficacy of OMC was determined in the hamster model of CDAD (ViviSource Laboratories, Inc., Waltham MA). Male LGV-Golden Syrian Hamsters were pretreated with a subcutaneous (SC) dose of clindamycin (10 mg/kg) and infected 24 hours later by oral gavage with a suspension of a 48 hour culture of Cd ATCC 43596, resulting in an inoculum of approximately 1.3 x 10^7 CFU/hamster. At 24 hours post infection, animals were treated PO with OMC (50 mg/kg/day), vancomycin (VAN 50 mg/kg/day) or vehicle for 5 days.

Results: The MIC_{90} for OMC against Cd was 0.06 mg/L by broth dilution and 0.12 mg/L by agar dilution, and OMC was more active than doxycycline (MIC_{90} = 0.5 mg/L by broth and 1 mg/L by agar). Against the infection model strain, OMC was as active as tigecycline, metronidazole, and VAN (MIC = 0.06 mg/L for all drugs). Day 2 post infection, 100% of OMC treated animals were alive, compared to 40% and 0% for VAN and vehicle control animals. For the OMC-treated animals, survival declined to 60% by Day 3 and remained at 60% until declining to 40% on Day 13 and to 0% by Day 16. Animals treated with VAN that survived the initial 2 days began to die by Day 11 (30% survival) and all animals succumbed to infection by Day 14. Overall, the median survival for OMC treated animals was 12 days compared to 2 days for VAN.

Conclusion: OMC exhibited potent *in vitro* activity against *C. difficile* and efficacy in the hamster model of CDAD and might offer potential for the treatment of *C. difficile* infections.

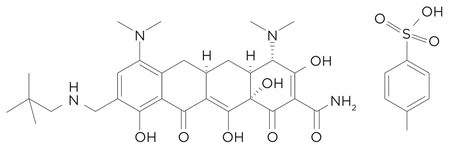
INTRODUCTION

C. difficile is the leading cause of healthcare-associated diarrhea and the most frequent cause of mortality associated with gastroenteritis in the healthcare system (Johanesen et al, 2015; Cohen et al, 2010). A recent surveillance study reported an estimated 450,000 infections and 29,000 deaths from *C. difficile* in the United States in 2011 (Lessa et al, 2015). Costs associated with *C. difficile* infection were estimated at $4.8 billion annually (Lessa et al, 2015). Treatment guidelines recommend a proactive approach to prevention as well as diagnosis and management of *C. difficile* infection (Cohen et al, 2010; Debast et al, 2014).

Most antibiotics have been implicated as a cause of *C. difficile* infections, although the risk with tetracyclines is among the lowest of antibiotic classes (Deshpande et al, 2013). Some evidence suggests that the tetracycline, doxycycline, may have protective effects against development of *C. difficile* infection (Turner et al, 2014).

Omadacycline is a first in class aminomethylcycline (Honeyman et al, 2015; **Figure 1**). Omadacycline exhibits a broad-spectrum of *in vitro* microbiological activity including Gram-positive and Gram-negative aerobes, anaerobes, and atypical bacteria (Macone et al, 2014; Flamm et al, 2015a, b, c; Dubois et al, 2015). Omadacycline has a half-life of approximately 17 hours after oral and IV administration, reaches peak plasma concentrations of 0.6 mg/L after oral administration, and exhibits low protein binding (21%). In earlier clinical studies, omadacycline was comparable to linezolid for treating patients with complicated skin infection. Phase 3 clinical development is ongoing with omadacycline as oral and intravenous (IV) monotherapy for the treatment of acute bacterial skin and skin structure infections (ABSSSI) and community acquired bacterial pneumonia (CABP). This study evaluated the microbiological activity of omadacycline *in vitro* against clinical isolates of *C. difficile* and determined efficacy in a hamster model of *C. difficile*.

Figure 1. Chemical Structure of Omadacycline



MATERIALS AND METHODS

In Vitro Activity

- Omadacycline was tested *in vitro* against 27 clinical isolates of *C. difficile* using broth and agar microdilution methods according to CLSI guidelines.
- Disks containing omadacycline 15 or 30 mcg were prepared.
- 96-well plates were filled with Wilkins-Chalgren broth containing final concentrations of 16 to 0.016 mg/L of each test compound.
- Plates were incubated for 48 hours under anaerobic conditions.
- Each test was run in duplicate.

Efficacy in Hamster Model of *C. difficile*

- The efficacy of omadacycline was determined in the hamster model of *C. difficile*-associated diarrhea (ViviSource Laboratories, Inc., Waltham MA).
- Male LGV-Golden Syrian Hamsters (Charles River Laboratories Inc., Wilmington, MA) weighing 80-100 g were used.
  - Hamsters were kept in a room maintained at 64-76° F (17.8-24.4° C) with humidity set at 40%-70%, and standard rodent diet and water were available *ad libitum*.
- Hamsters were pretreated with a subcutaneous (SC) dose of clindamycin (10 mg/kg) 24 hours prior to injection.
- *C. difficile* ATCC 43596 was obtained from American Type Culture Collection, Manassas, VA.
- *C. difficile* ATCC 43596 was cultured from freezer stocks under anaerobic conditions on Brucella agar with 5% sheep blood.
- Hamsters were infected 24 hours later by oral gavage (10 mL/kg) with a suspension of a 48 hour culture of *C. difficile* ATCC 43596, resulting in an inoculum of approximately 1.3 x 10^7 CFU/hamster.
- At 24 hours post infection, groups of animals (N=10) received an oral dose of omadacycline 50 mg/kg/day, vancomycin 50 mg/kg/day or vehicle (sterile water) for 5 days.
- Animals were observed daily to assess general health; body weight was recorded at least 3 times weekly.
- *In vitro* activity also was determined for clindamycin, tigecycline, vancomycin, and metronidazole.

Data Analysis

- Percent survival for each group was determined for up to 21 days post infection.
- Kaplan-Meier survival analysis was performed with a staircase plot.
- Using a Log Rank analysis of data, p-values, significant difference in curves, and median survival were determined.

RESULTS

- The MIC_{90} for omadacycline against *C. difficile* was 0.06 mg/L by broth dilution and 0.12 mg/L by agar dilution (**Table 1**).
- Omadacycline was more active than doxycycline (MIC_{90} = 0.5 mg/L by broth and 1 mg/L by agar).

Table 1. Minimum Inhibitory Concentration For Omadacycline and Comparators Against *C. difficile* Strains (N=27) by Broth and Agar Dilution Methods

	Minimum Inhibitory Concentration (mg/L)					
	Broth Microdilution			Agar Microdilution		
Drug	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Omadacycline	0.06–0.12	0.06	0.06	0.06–0.12	0.12	0.12
Cefotaxime	4–>128	64	128	4–>128	>64	>128
Doxycycline	0.015–0.5	0.03	0.5	0.03–2	0.03	1
Amox/Clav.	0.12–0.5	0.25	0.5	0.25–1	0.05	1
Metronidazole	0.12–1	0.12	0.5	0.06–0.5	0.12	0.25
Imipenem	0.5–8	4	8	0.5–8	4	8
Clindamycin	0.12–>16	4	>16	0.12–>16	8	>16

- Against the infection model strain, omadacycline was as active as tigecycline, metronidazole, and vancomycin (MIC_{90} = 0.06 mg/L for all drugs), while clindamycin exhibited no activity (**Table 2**).

Table 2. Minimum inhibitory Concentration (MIC_{90}) For Omadacycline and Tested Compounds in the infection Model

	MIC_{90} (mg/L)	
	C. difficile ATCC 43596	
Compound	Plate A	Plate B
Omadacycline	0.06	0.06
Tigecycline	0.06	0.06
Metronidazole	0.06	0.06
Clindamycin	>32	>32
Vancomycin	0.06	0.06

Survival

- Day 2 post infection, 100% of omadacycline treated animals were alive, compared to 40% and 0% for vancomycin and vehicle control animals (**Figure 2**).
- Hamsters receiving only clindamycin pre-treatment demonstrated 100% survival at Day 2 post infection.
- For omadacycline -treated animals, survival declined to 60% by Day 3 and remained at 60% until declining to 40% on Day 13 and to 0% by Day 16.
- Animals treated with vancomycin that survived the initial 2 days began to die by Day 11 (30% survival) and all animals succumbed to infection by Day 14.
- Overall, the median survival for omadacycline-treated animals was 12 days compared to 2 days for vancomycin and 4 days for clindamycin pre-treatment (**Table 3**).

Figure 2. Kaplan-Meier analysis of Percent Survival of Hamsters Infected With *C. difficile* After Treatment With Omadacycline and Comparators.

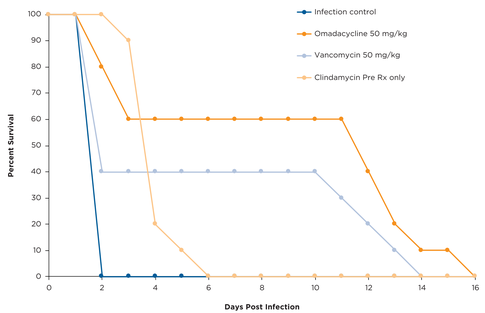


Table 3. Median Survival For Hamsters After Treatment With Omadacycline and Comparators

Test Compound	Median Survival (days)	p-value*
Omadacycline	12	0.0004
Vancomycin	2	0.0293
Clindamycin	4	<0.0001

*Kaplan-Meier analysis using log rank test.

SUMMARY AND CONCLUSIONS

- Omadacycline exhibited potent *in vitro* activity against *C. difficile* that was similar to comparators.
- Omadacycline demonstrated efficacy in the hamster model of *C. difficile*-associated diarrhea that was superior to vancomycin.
- Omadacycline might offer potential for the treatment of *C. difficile* infections.

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