In Vitro Bacterial and Intracellular Activity of Omadacycline Against *Legionella pneumophila*

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Abstract

Background: **Omadacycline (OMC)** is the first aminomethylcycline in late stage clinical development for community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infection (ABSSI) as once-daily oral and IV formulations. *In vitro* bacterial activity and intracellular activities using human monocytes against a variety of *L. pneumophila* serogroup 1 were investigated.
Methods: The *in vitro* activity of **OMC** was compared with that of doxycycline (DO), azithromycin (AZ), erythromycin (ER), levofloxacin (LE), and moxifloxacin (MO) against a total of 90 *L. pneumophila* serogroup 1 by microdilution procedure using buffered yeast extract broth containing *Legionella* growth supplement (BYE). A pre-test to determine if antibiotic activity was impacted artificially by *Legionella* supplement or iron was done by testing 3 ATCC quality control isolates on BYE, BYE without iron, and cation-adjusted Mueller-Hinton Broth (MH). The intracellular activity of **OMC** was compared against a total of 3 ER-resistant and 2 ER-susceptible strains of *L. pneumophila* serogroup 1. The intracellular activity was determined by exposing human monocytes, U937 cell line, with intracellular *L. pneumophila* to antibiotic at 1X the extracellular MIC of each strain during either 2 or 6 days of exposure. Counts of CFU/mL were performed daily in duplicate using the BYE agar with charcoal.
Results: Against tested *L. pneumophila* serogroup 1, the $MIC_{50/90}$ of MO, LE, **OMC**, AZ, ER, and DO was 0.008/0.016, 0.016/0.016, **0.25/0.25**, 0.12/0.5, 0.25/1 and 1/1 mg/L in BYE, respectively. Pilot tests suggested that the MIC values of **OMC** and DO obtained in BYE for *L. pneumophila* may be artificially elevated (5- to 7-fold increase) due to the media effects. A significant reduction of more than 3 $\log_{10}$ CFU/mL or 99.9% of ER-susceptible or ER-resistant *L. pneumophila* grown in monocytes was observed after 4 to 6 days of continuous exposure to **OMC** at 1X the MIC. A regrowth of *L. pneumophila* in monocytes was observed after 1 day of ER exposure, after 3 days of AZ and DO exposure, and after 4 days of LE exposure, however this regrowth was not observed with **OMC** and MO. After drug wash-out at day 2 of drug exposure, **OMC** followed by MO, DO, LE, and AZ, slowed substantially the regrowth of *L. pneumophila* tested strains in human monocytes, whereas a rapid regrowth occurred for ER-treated culture.
Conclusions: This data demonstrating good bacterial activity and human monocytes penetration, suggest that **OMC** may have use in infections caused by *L. pneumophila* and highlights the potential utility of this oral and IV agent for the treatment of CABP.

Introduction

Omadacycline is the first aminomethylcycline to be developed as a once daily, oral and IV treatment of Acute Bacterial Skin and Skin Structure Infection (ABSSSI) and Community-Acquired Bacterial Pneumonia (CABP). The Phase 3 development program has now been initiated. **Omadacycline** has excellent activity against the primary pathogens associated with ABSSSI and CABP, including antibiotic resistant organisms, including *S. aureus*, β-hemolytic *streptococci*, *S. pneumoniae*, *H. influenzae*, *Legionella* and *C. pneumoniae*.

Objective

The goal of this study was to investigate the bacterial and intracellular activity of **omadacycline** against *Legionella pneumophila.* We determined the minimum inhibitory concentration (MIC) and the intracellular human monocytes activity of **omadacycline**, doxycycline, azithromycin, erythromycin, levofloxacin and moxifloxacin.) against a variety of *Legionella pneumophila* serogroup 1 isolated from nosocomial or acquired respiratory tract infections.

Materials and Methods

Strains
A total of 90 strains of *L. pneumophila* serogroup 1 isolated from 1995 to 2014 were collected from mostly nosocomial or acquired respiratory tract sources and were identified by standard methods such as described by Versalovic et al. (1).
Determination of MICs
MICs were determined using the CLSI broth medium microdilution method using microdilution plating of the organisms onto a series of broth medium microplates of increasing concentrations from 0.004 mg/L to 128 mg/L (2, 3). Buffered Yeast extract (BYE) was used as the medium against *Legionella* strains. *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* ATCC27853 and *L. pneumophila* ATCC33152 were included as controls.
Growth conditions
S. aureus ATCC29213, *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *L. pneumophila* ATCC33152 were tested in a media testing study comparing the activities of antibiotics in cation adjusted Mueller Hinton Broth (MH), standard BYE, and modified BYE ("Mod BYE"; lacking ferric pyrophosphate). Only representative data for *E. coli* and *L. pneumophila* is shown in Table 1 due to similar data obtained by *S. aureus* and *P. aeruginosa*.
Determination of Intracellular Human Monocytes Activity
The intracellular activity of **omadacycline** was compared against a total of 3 ER-resistant and 2 ER-susceptible strains of *L. pneumophila* serogroup 1. The in vitro method using the mononuclear cells described by M.A Horwitz (4) was performed using 48 wells microplates. RPMI 1640 medium (with 10% heat-inactivated foetal bovine serum), mononuclear cells (U-937; 1-2 $X10^6$ cells/ ml) and *Legionella* inoculum (10^4-10^5 CFU/ml) have been used. After a 1 hour's exposure in a shaking incubator, the infected cultures were maintained under stationary conditions thereafter for 7 days at 37°C in 5% CO_2 and 95% air. After 24h (Day 1), the infected cultures were washed (three times). The antimicrobials (1 X MIC) have been added and cultures were incubated for 2 days. After 72h (Day 3), cultures have been washed (three times) and have been split into two groups; one group with the same antibiotic as before washing and the other group without antibiotic (to observe potential intracellular post-antibiotic effect) for both a 4 additional days of incubation. Counts of CFU/mL were performed daily in duplicate using the BYE agar with charcoal.

Results

Table 1. Susceptibility of *Legionella pneumophila serogroup 1* (from 1995 to 2014)

Organism (no. tested)	Collection Date	Antibiotic	MIC (mg/L)		
			Range	MIC50	MIC90
Legionella pneumophila serogroup 1 (90)	From 1995-2014	**Omadacycline**	**0.06-0.5**	**0.25**	**0.25**
		Doxycycline	0.5-1	1	1
		Azithromycin	0.016-0.5	0.12	0.5
		Erythromycin	0.06-2	0.25	1
		Levofloxacin	≤0.004-0.03	0.016	0.016
		Moxifloxacin	≤0.004-0.06	0.008	0.016

Results continued

Table 2. Media Study: Susceptibility of QC strain: *E. coli* ATCC25922 & *L. pneumophila* ATCC33152

QC Strain & Incubation time	Media tested	Antibiotic MIC (mg/L)			
		Omadacycline	Doxycycline	Azithromycin	Erythromycin
E. coli ATCC25922					
24 hours	Cation adjusted M-H	**1**	2	>128	>128
	Modified BYE	**16**	0.5	>128	>128
	BYE	**32**	64	>128	>128
48 hours	Cation adjusted M-H	Not Done	Not Done	Not Done	Not Done
	Modified BYE	**16**	1	>128	>128
	BYE	**128**	>128	>128	>128
Expected MIC range	Cation adjusted M-H	**0.25-2***	0.5-2*	Unknown	Unknown
L pneumophila ATCC33152					
24 hours	Cation adjusted M-H	No Growth	No Growth	No Growth	No Growth
	Modified BYE	No Growth	No Growth	No Growth	No Growth
	BYE	No Growth	No Growth	No Growth	No Growth
48 hours	Cation adjusted M-H	No Growth	No Growth	No Growth	No Growth
	Modified BYE	No Growth	No Growth	No Growth	No Growth
	BYE	**0.25**	1	0.06	0.25

* Expected MIC Range with Cation adjusted Mueller-Hinton, data obtained from CLSI

Figure 1: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 (all tested strains (5 strains)) with antibiotic (1XMIC) from Day 1 until Day 7 of incubation

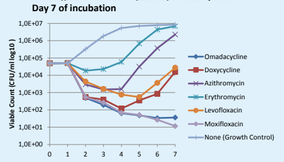


Figure 2: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 (all tested strains (5 strains)) without antibiotic (1XMIC) after day 3 of incubation

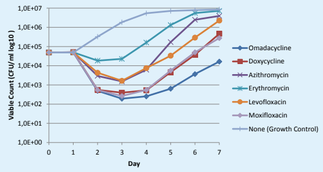


Figure 3: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 ER-susceptible (2 strains) with antibiotic (1XMIC) from Day 1 until day 7 of incubation

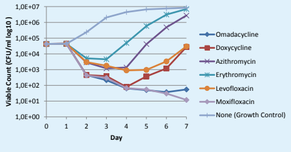


Figure 4: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 ER-susceptible (2 strains) without antibiotic (1XMIC) after day 3 of incubation

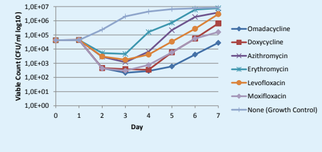


Figure 5: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 ER-resistant (3 strains) with antibiotic (1XMIC) from Day 1 until day 7 of incubation

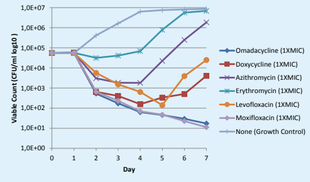
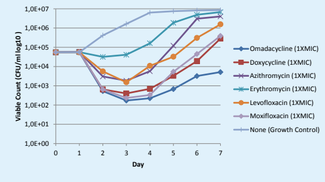


Figure 6: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 ER-resistant (3 strains) without antibiotic (1XMIC) after day 3 of incubation



Discussion

- **Omadacycline** and doxycycline MICs were 5-7-dilution higher in BYE broth with iron, compared to broth without the iron supplement, indicating that the MIC's of **omadacycline** may be artificially elevated *in vitro* due to media effects.
- Against *L. pneumophila* serogroup 1 which is the most resistant *Legionella* serogroup to erythromycin and the most usual *Legionella* serogroup isolated from respiratory tract infections, **omadacycline** (MIC_{90}=0.25 mg/L) is more active than doxycycline (MIC_{90}=1 mg/L), erythromycin (MIC_{90}=1 mg/L) and azithromycin (MIC_{90}=0.5 mg/L) that are the most commonly used drugs for the treatment of Legionellosis.
- Moxifloxacin and levofloxacin are the most active compounds tested followed by **omadacycline**.
- Compared to azithromycin and erythromycin, an important reduction of 3 $\log_{10}$ CFU/ml or 99.9% of *L. pneumophila* serogroup 1 grown in macrophages was reached only by **omadacycline** and moxifloxacin after 3 days of antibiotic exposure.
- Unlike **omadacycline** and moxifloxacin, a significant regrowth of *L. pneumophila* ER-susceptible or ER-resistant strain in macrophages was observed after 2 days, 3 days, 3 days and 4 days of antibiotic exposure respectively by erythromycin, azithromycin, doxycycline and levofloxacin.
- Even if we observed with **omadacycline** a regrowth of the intracellular *L. pneumophila* after drug wash out at day 3 of incubation, erythromycin, azithromycin and levofloxacin allowed a more important regrowth in contrast to substantially delayed regrowth observed with **omadacycline** followed by moxifloxacin and doxycycline.

Conclusion

Based on the *in vitro* results of this study, **omadacycline** exhibits potent extracellular and intracellular activity against *L. pneumophila* serogroup 1 and warrants further study as a potential antimicrobial agent for the treatment of pneumonia caused by *L. pneumophila*..

References

1. Versalovic et al., Manual of Clinical Microbiology, 10rd ed., 2011, A.S.M.
2. Performance standards for antimicrobial susceptibility testing; 22th Informational Supplement; M100-S22, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, January 2012)
3. Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard 9th edition, M07-A9, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, 2012)
4. Horwitz, *Legionella*, Proceedings of the 2nd International Symposium, American Society for Microbiology, 1984, 159-166