

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 log₁₀ 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 (ABSSI) 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 ABSSI 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 X10⁶ cells/ml) and *Legionella* inoculum (10⁴-10⁵ 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% CO₂ 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
<i>Legionella pneumophila</i> 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
<i>E. coli</i> ATCC25922	24 hours	1 16 32	2 0.5 64	>128 >128 >128	>128 >128 >128
	48 hours	Not Done 16 128	Not Done 1 >128	Not Done >128 >128	Not Done >128 >128
	Expected MIC range	Cation adjusted M-H	0.25-2*	0.5-2*	Unknown
<i>L. pneumophila</i> ATCC33152	24 hours	No Growth No Growth No Growth	No Growth No Growth No Growth	No Growth No Growth No Growth	No Growth No Growth No Growth
	48 hours	No Growth No Growth	No Growth No Growth	No Growth No Growth	No Growth No Growth
		Cation adjusted M-H Modified BYE 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

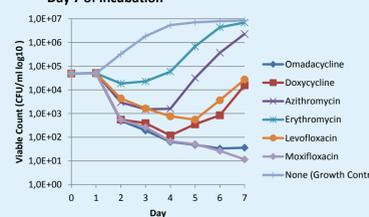


Figure 3: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 ER-susceptible (2 strains) with antibiotic (1XMIC) from Day 1 until day 7 of incubation

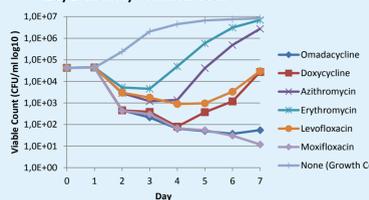


Figure 5: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 ER-resistant (3 strains) with antibiotic (1XMIC) from Day 1 until day 7 of incubation

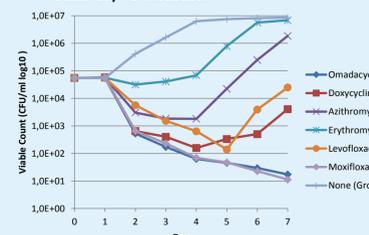


Figure 2: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 (all tested strains (5 strains)) without antibiotic (1XMIC) after day 3 of incubation

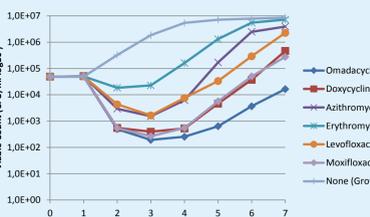


Figure 4: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 ER-susceptible (2 strains) without antibiotic (1XMIC) after day 3 of incubation

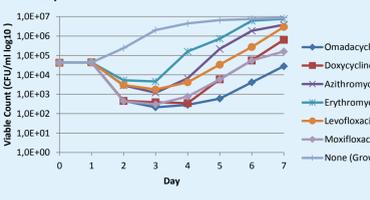
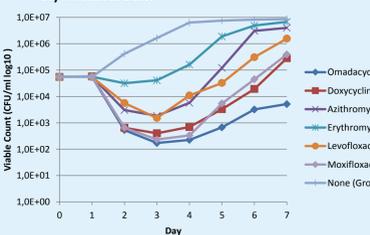


Figure 6: *In vitro* intracellular activity of omadacycline and comparators against *L. pneumophila* Serogroup 1 ER-resistant (3 strains) without antibiotic (1XMIC) after day 3 of incubation



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** (MIC₉₀=0.25 mg/L) is more active than doxycycline (MIC₉₀=1 mg/L), erythromycin (MIC₉₀=1 mg/L) and azithromycin (MIC₉₀=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 log₁₀ 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

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- Performance standards for antimicrobial susceptibility testing; 22th Informational Supplement; M100-S22, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, January 2012)
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- Horwitz, *Legionella*, Proceedings of the 2nd International Symposium, American Society for Microbiology, 1984, 159-166