Abstract #1504
Poster: MONDAY-551

In Vitro Intracellular Activity of Omadacycline Against *Legionella pneumophila*

J. DUBOIS[1]*, M. DUBOIS[1], J. –F. MARTEL[1], S.K. TANAKA[2]

[1] M360, Sherbrooke, Qué., Canada, [2] Paratek Pharmaceuticals, Boston, MA, USA

ASM MICROBE 2016, Boston, Massachusetts
Jacques Dubois Ph.D.
M360, Sherbrooke, Québec, Canada
819.571.4366
jdubois@m360.ca

Abstract

Background: Omadacycline (OMC) is the first once-daily, oral and IV aminomethylcycline in late stage clinical development for serious community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infection (ABSSI). *In vitro* bacterial activity and intracellular activities using human monocytes against a variety of *L. pneumophila* serogroup one (1) were investigated.
Methods: The intracellular activity of **OMC** was compared with that of doxycycline (DO), moxifloxacin (MO) and azithromycin (AZ) against a total of three (3) erythromycin-resistant and two (2) erythromycin-susceptible strains of *L. pneumophila* serogroup 1. The minimal extracellular concentration inhibiting the intracellular multiplication of *L. pneumophila* (MIEC) was determined by exposing human monocytes, U937 cell line, with intracellular *Legionella* to antibiotic at the 1X, 1/2X, 1/4X, 1/8X or 1/16X the extracellular MIC of each strain during 4 days exposure. Counts of CFU/mL were performed daily in triplicate using the Buffer Yeast Extract agar with charcoal.
Results: All tested strains of *L. pneumophila* (5/5 strains) had a MIC of 0.25 mg/L to **OMC.** A mean reduction of ≥50% of *L. pneumophila* grown in human monocytes at Day 3 or Day 5 of **OMC** exposure, was achieved at a MIEC of 0.06 mg/L or at MIEC/MIC ratio of 0.24 (1/4XMIC). An intracellular activity at Day 3 or at Day 5 of **OMC** exposure with a reduction (≥50%) of *L. pneumophila* grown in macrophages and with a MIEC/MIC ratio of 0.12 (1/8XMIC) was observed with only 1 erythromycin-resistant strains of *L. pneumophila*. In this study, the MIEC/MIC ratio of **OMC** (0.24 or 1/4XMIC) was lower than the MIEC/MIC ratio of doxycycline (0.5 or 1/2XMIC), moxifloxacin (0.5 or 1/2XMIC), and azithromycin (1 or 1XMIC).
Conclusions: These data, demonstrating robust bacterial activity and human monocyte penetration, highlight the potential utility of **OMC** as an effective oral and IV antibiotic for the treatment of CABP caused by *L. pneumophila*.

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 is ongoing. **Omadacycline** has excellent activity against the primary pathogens associated with ABSSSI and CABP, including antibiotic resistant organisms such as *S. aureus*, β-hemolytic *streptococci*, *S. pneumoniae* and *H. influenzae*, *Legionella* ssp. and *C. pneumoniae*.

Objective

The goal of this study was to investigate the intracellular activity of **omadacycline** against *Legionella pneumophila.* We determined the minimal extracellular concentration inhibiting the intracellular multiplication of *L. pneumophila* (MIEC) of **omadacycline**, doxycycline, azithromycin and moxifloxacin against a variety of isolates of *Legionella pneumophila* serogroup 1, isolated from nosocomial or acquired respiratory infections.

Materials and Methods

Strains

A total of 5 strains of *L. pneumophila* serogroup 1 (3 Erythromycin (ER)-resistant and 2 ER-susceptible strains) 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 Intracellular Human Monocytes Activity (MIEC)

The *in vitro* method using the mononuclear cells described by M.A Horwitz (2) was performed using 48 well 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) were used. After a 1 hour exposure in a shaking incubator, the infected cultures were maintained under stationary conditions thereafter for 6 days at 37ºC in 5% CO_2 and 95% air. After 24h of incubation (or Day 1 of antibiotic exposure) and after 72h of incubation (or Day 3 of antibiotic exposure), the infected cultures were washed (three times). At Day 1 and Day 3 of antibiotic exposure, each strain was exposed to antimicrobial concentration of 1, 1/2-1/4- 1/8 or 1/16 times the MIC required for **omadacycline** and comparators to determine the precise MIEC. The cultures were incubated during 4 days of antibiotic exposure. Counts of CFU/mL were performed daily in triplicate using the BYE agar with charcoal. The MIEC was defined as the lowest MIC concentration that was able to produce an intracellular reduction of more than 50% (CFU/ml) of *L. pneumophila.* The MIEC was calculated at Day 3 and Day 5 of antibiotic exposure.

Results

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

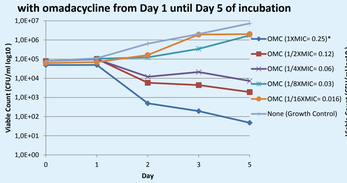


Figure 2: ***In vitro*** **intracellular activity (MIEC) against *L. pneumophila* Serogroup 1 (all tested strains (5 strains)) with doxycycline from Day 1 until Day 5 of incubation**

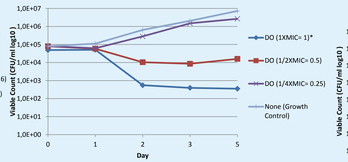


Figure 3: ***In vitro*** **intracellular activity (MIEC) against *L. pneumophila* Serogroup 1 (all tested strains (5 strains)) with azithromycin from Day 1 until Day 5 of incubation**

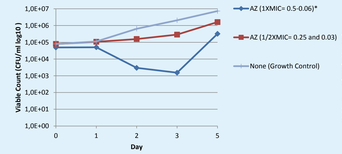
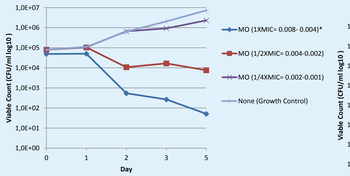


Figure 4: ***In vitro*** **intracellular activity (MIEC) against *L. pneumophila* Serogroup 1 (all tested strains (5 strains)) with moxifloxacin from Day 1 until Day 5 of incubation**



Results continued

Table 1. MIC, MIEC and MIC/MIEC ratio of *Legionella pneumophila (5 tested strains)*:

Organism #Strain number	Antibiotic	MIC (mg/L)	MIEC[a] (mg/L)	MIEC/MIC[b]	MIEC[c] (mg/L)	MIEC/MIC[d]
L. pneumophila Serogroup 1 ATCC33152 Erythro-susceptible	**Omadacycline**	0,25	0,06	0,24	0,06	0,24
	Doxycycline	1	0,5	0,5	0,5	0,5
	Azithromycin	0,06	0,06	1	ND	ND
	Moxifloxacin	0,008	0,004	0,5	0,004	0,5
L. pneumophila Serogroup 1 #7 Erythro-susceptible	**Omadacycline**	0,25	0,06	0,24	0,06	0,24
	Doxycycline	1	0,5	0,5	0,5	0,5
	Azithromycin	0,06	0,06	1	ND	ND
	Moxifloxacin	0,004	0,002	0,5	0,002	0,5
***L. pneumophila* Erythro-susceptible (2 strains tested)**	**Omadacycline**	0,25	0,06	0,24	0,06	0,24
	Doxycycline	1	0,5	0,5	0,5	0,5
	Azithromycin	0,06	0,06	1	ND	ND
	Moxifloxacin	0,008 and 0,004	0,004 and 0,002	0,5	0,004 and 0,002	0,5
L. pneumophila Serogroup 1 #18 Erythro-resistant	**Omadacycline**	0,25	0,06	0,24	0,03	0,12
	Doxycycline	1	0,5	0,5	0,5	0,5
	Azithromycin	0,5	0,5	1	ND	ND
	Moxifloxacin	0,008	0,004	0,5	0,004	0,5
L. pneumophila Serogroup 1 #20 Erythro-resistant	**Omadacycline**	0,25	0,06	0,24	0,06	0,24
	Doxycycline	1	1	1	1	1
	Azithromycin	0,5	0,5	1	0,5	1
	Moxifloxacin	0,008	0,008	1	0,004	0,5
L. pneumophila Serogroup 1 #22 Erythro-resistant	**Omadacycline**	0,25	0,03	0,12	0,03	0,12
	Doxycycline	1	0,5	0,5	0,5	0,5
	Azithromycin	0,5	0,5	1	ND	ND
	Moxifloxacin	0,008	0,004	0,5	0,004	0,5
***L. pneumophila* Erythro-resistant (3 strains tested)**	**Omadacycline**	0,25	0,06	0,24	0,06	0,24
	Doxycycline	1	0,5	0,5	1	1
	Azithromycin	0,5	0,5	1	0,5	1
	Moxifloxacin	0,008	0,004	0,5	0,004	0,5
***L. pneumophila* all tested strains (5 strains tested)**	**Omadacycline**	0,25	0,06	0,24	0,06	0,24
	Doxycycline	1	0,5	0,5	0,5	0,5
	Azithromycin	0,5 and 0,06	0,5 and 0,06	1	ND	ND
	Moxifloxacin	0,008 and 0,004	0,004 and 0,002	0,5	0,004 and 0,002	0,5

[a]MIEC after 2 days of drug exposure: [c]MIEC after 4 days of drug exposure
[b]MIEC/MIC ratio after 2 days of drug exposure: [d]MIEC/MIC ratio after 4 days of drug exposure; ND= Not Done

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

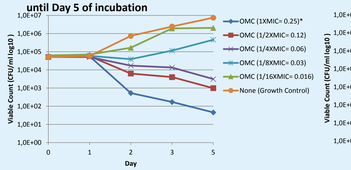


Figure 6: ***In vitro*** **intracellular activity (MIEC) against *L. pneumophila* Serogroup 1 (ER-resistant strains (3 strains)) with doxycycline from Day 1 until Day 5 of incubation**

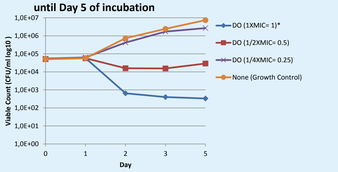


Figure 7: ***In vitro*** **intracellular activity (MIEC) against *L. pneumophila* Serogroup 1 (ER-resistant strains (3 strains)) with azithromycin from Day 1 until Day 5 of incubation**

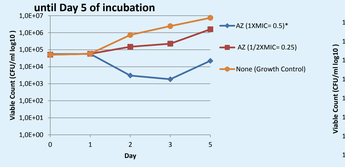


Figure 8: ***In vitro*** **intracellular activity (MIEC) against *L. pneumophila* Serogroup 1 (ER-resistant strains (3 strains)) with moxifloxacin from Day 1 until Day 5 of incubation**

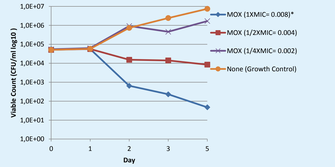


Figure 9: ***In vitro*** **intracellular activity (MIEC) against *L. pneumophila* Serogroup 1 (ER-susceptible strains (2 strains)) with omadacycline from Day 1 until Day 5 of incubation**

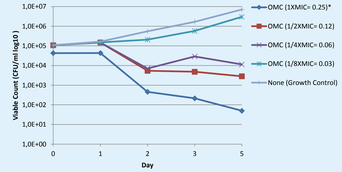
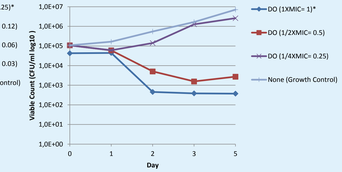


Figure 10: ***In vitro*** **intracellular activity (MIEC) against *L. pneumophila* Serogroup 1 (ER-susceptible strains (2 strains)) with doxycycline from Day 1 until Day 5 of incubation**



Discussion

- Intracellular activity with a mean reduction (≥50%) of *L. pneumophila* grown in macrophages was easily detected at Day 3 or at Day 5 of **omadacycline** exposure, with a MIEC/MIC ratio of 0.24 (1/4XMIC) obtained by the 5 tested strains of *L. pneumophila* with a MIC of 0.25 mg/L.
- This MIEC result (0.06 mg/L) or MIEC/MIC ratio (0.24 (1/4XMIC)) of **omadacycline** was also observed at Day 3 or at Day 5 of **omadacycline** exposure, against most *L. pneumophila* tested strains (4/5) including erythromycin-susceptible or erythromycin-resistant strains.
- Only 1 tested strain of *L. pneumophila* showed an important reduction (≥50%) of *L. pneumophila* grown in macrophages at Day 3 or at Day 5 of **omadacycline** exposure, with a MIEC/MIC ratio of 0.12 ((1/8XMIC).
- At Day 3 of antibiotic exposure, the MIEC's (with a ≥50% reduction of *L. pneumophila* grown in macrophages) of doxycycline, azithromycin moxifloxacin against all *L. pneumophila* tested strains were respectively equal to 0.5 mg/L for 80% (4/5), equal or less than 0.5 mg/L for 100% (5/5) and equal or less than 0.004 mg/L for 80% (4/5) of tested strains.
- At Day 5 of antibiotic exposure, the MIEC's of doxycycline and moxifloxacin against *L. pneumophila* tested strains were similar to MIEC's obtained at Day 3 of antibiotic exposure.
- Even if the MIEC results of doxycycline, moxifloxacin and azithromycin were lower or higher than the MIEC's results of **omadacycline**, the MIEC/MIC ratio of **omadacycline** (0.24 or 1/4XMIC) was consistently lower than the MIEC/MIC ratio of doxycycline (0.5 or 1/2XMIC), moxifloxacin (0.5 or 1/2XMIC) and azithromycin (1 or 1XMIC).

Conclusion

Based on the *in vitro* results of this study, **omadacycline** exhibits robust bacterial activity and human monocyte penetration highlighting the potential utility of **omadacycline** as an effective oral and IV antibiotic for the treatment of CABP caused by *L. pneumophila.*

References

1. Versalovic et al., Manual of Clinical Microbiology, 10rd ed., 2011, A.S.M.
2. Horwitz, *Legionella*, Proceedings of the 2nd International Symposium, American Society for Microbiology, 1984, 159-166.
3. * 1XMIC curve has been obtained from: Dubois et al., *In vitro* bacterial and intracellular activity of Omadacycline against *Legionella pneumophila*; ECCMID 2016, Poster P1323.