In Vitro Activity of Omadacycline (PTK796) in Broth, Broth plus Lung Surfactant or Human Serum

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Poster – P1141

ABSTRACT

Background
Determine the effects of lung surfactant and human serum on the *in vitro* activity of omadacycline (OMC)

Methods
MICs were performed in three systems. Cation-adjusted Mueller Hinton broth was used according to standard CLSI microdilution procedures, in addition to broth enriched with 1% bovine surfactant, and 25%-50% human serum. The strains included were clinical Gram-positive, (*S. aureus, S. pneumoniae)*, also Gram-negative, *(E. coli* and *H. influenzae)*, selected to include a range of susceptibilities, and appropriate ATCC controls. Doxycycline (DOXY) and daptomycin (DAPTO) were tested as comparators.

Results
MIC ranges in all three systems are shown below (Table 1).

Table 1

Organism	N	Condition	Range MIC mg/L		
			OMC	DOXY	DAPTO
S. aureus	6	Broth	0.125-0.5	≤0.06-4	0.25
		Surfactant	0.125-0.5	≤0.06-4	16
		Serum (50%)	0.25-0.5	0.5-16	2-4
S. pneumoniae	3	Broth	0.03-0.06	0.03-4	0.25
		Surfactant	0.03-0.06	0.03-4	4-16
		Serum (50%)	0.03	0.125-4	0.5-1
E.coli	6	Broth	0.5-4	0.5-32	N/A
		Surfactant	0.5-2	0.5-64	N/A
		Serum (50%)	0.25-2	1- >64	N/A
H. influenzae	6	Broth	0.5-4	0.5-4	N/A
		Surfactant	0.5-4	0.25-4	N/A
		Serum (50%)	0.5-4	1-8	N/A

Conclusions
MICs of omadacycline did not increase with the addition of surfactant or serum for either Gram-positive or Gram-negative organisms. The *in vitro* activity of DAPTO was markedly affected by surfactant, as well as serum reflecting its high protein binding character. DOXY activity was not affected by surfactant, but exhibited some decreases in activity in the presence of serum. The inhibition of activity of DAPTO by lung surfactant has been suggested as the explanation for its lack of efficacy in pneumonia. (Silverman, JA et al, JID 191:2149-2152. 2005). In contrast, OMC was not affected by surfactant. This finding supports the potential use of OMC in treating pneumonia caused by susceptible bacteria.

INTRODUCTION

Omadacyline (OMC, formerly PTK796) is a novel aminomethylcycline with a spectrum of activity consistent with its potential utility in the treatment of community-acquired pneumonia. The failure of daptomycin in pneumonia has been attributed to interactions of daptomycin with lung surfactant, compromising its ability to kill bacteria in lung tissue. It has also been known that strong interactions with serum can adversely affect efficacy and this is also demonstrable *in vitro*. In order to determine the effects of lung surfactant and human serum on the *in vitro* activity of omadacycline, MICs were done in three systems. Cation-adjusted Mueller Hinton broth or *Haemophilus* test media was used according to standard CLSI microdilution procedures, in addition to broth enriched with 1% surfactant, and 25%-50% human or mouse serum. Appropriate media supplementation was also done for fastidious organisms. The clinical isolates included in this study represented potential human lung pathogens in addition to appropriate ATCC Quality Control strains (CLSI).

MICROBIOLOGY METHODS

Materials

- Cation-adjusted Mueller Hinton II broth (CAMHB, BBL)
- Compounds
  - Omadacycline (P000949-147, Paratek Pharmaceuticals, Inc. Boston MA)
  - Doxycycline (P000001-9, Hovione, Portugal)
  - Daptomycin (Cubist Pharmaceuticals, Lexington MA)
- Blood agar and chocolate agar plates (Northeast labs, NEL, Waterville ME)
- 5ml Mueller Hinton broth (MH, NEL)
- HTM broth (Remel, Thermofisher Scientific, Lenexa, KS))
- Human serum (Atlanta Biologicals, Lawrenceville, GA)
- Mouse serum (Equitech-Bio Inc. Kerrville, TX)
- Bovine lung surfactant (Survanta, Ross Division of Abbott Nutrition, Columbus OH)
- Lysed horse blood (Rockland Immunochemicals Inc. Gilbertsville, PA)

Bacterial Strains

Clinical strains of Gram-positive and Gram-negative isolates (tetracycline-susceptible and resistant) were obtained from the following sources: Clinical Microbiology Institute, Wilsonville, OR, Glaxo Smith Kline, Collegeville, PA, University of California at Los Angeles Medical Center, Los Angeles, CA, University of Wisconsin Hospitals and Clinics, Madison, WI, and VA Medical Center, Boston MA.

- 5 *S. aureus*
- 2 *S. pneumoniae*
- 5 *H. influenzae*
- 5 *E. coli*

Quality control strains were obtained from the American type Culture Collection (ATCC, Manassas, VA).

- *S. aureus* ATCC 29213
- *S. pneumoniae* ATCC 49619
- *E. coli* ATCC 25922
- *H. influenzae* ATCC 49247

All isolates were stored at –80°C in broth plus 20% glycerol. Horse blood supplementation was used for fastidious organisms. Isolates were subcultured twice onto appropriate solid media such as, tryptic soy agar with 5% sheep blood or chocolate agar, prior to MIC testing.

MIC Testing

Microdilution MICs were performed according to CLSI guidelines (Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Seventh edition, 2006) using cation-adjusted Mueller Hinton broth (CAMHB) or *Haemophilus* test medium (HTM). Lysed horse blood was used to supplement the inoculum of *S. pneumoniae.* HTM was used for *H. influenzae.*

Antibiotics were prepared according to manufacturer instructions. Stock solutions of compounds were serially diluted in CAMHB or HTM utilizing a Tecan Genesis workstation (Tecan, Research Triangle Park, NC).

Organisms were grown in MH broth to match the density of a 0.5 McFarland standard. Turbidity was measured using a nephelometer (Microscan turbidity meter, Siemens, Deerfield, IL). Alternatively, a direct suspension of organisms was made for inoculation. Activity in the presence of serum (heat inactivated, 56ºC, 45min) or surfactant were determined by preparing plates containing final concentrations in CAMHB or HTM broth of either 25% heat inactivated serum for *S. pneumoniae* or 50% for other organisms, or 1% bovine surfactant.

MICs were recorded as the lowest concentration that inhibited visible growth after overnight incubation.

RESULTS

Susceptibility testing results are shown in Tables 2-5 for *S. aureus, S pneumoniae, E. coli* or *H. influenzae.*

- Omadacycline MICs were not affected by the addition of serum or lung surfactant to the cation-adjusted Mueller Hinton broth or Haemophilus test medium.
- Doxycycline MICs increased with the addition of serum.
- Daptomycin MICs increased in the presence of serum or lung surfactant.

Table 2. Effect of surfactant or serum on the *in vitro* activity of omadacycline, doxycycline, and daptomycin against clinical isolates of *S. aureus* including MRSA.

Organism	Condition	MIC (mg/L)		
		OMC	DOXY	DAPTO
S. aureus ATCC 29213	Broth	0.25	0.125	0.25
	Surfactant (1%)	0.5	0.25	16
	Human Serum (50%)	0.5	1.0	2.0
MRSA PBS 487	Broth	0.25	4.0	0.25
	Surfactant (1%)	0.25	4.0	16
	Human Serum (50%)	0.5	16	4.0
MRSA PBS 1313	Broth	0.125	2.0	0.25
	Surfactant (1%)	0.5	4.0	16
	Human Serum (50%)	0.5	8.0	2.0
S. aureus PBS 863	Broth	0.25	≤0.06	0.25
	Surfactant (1%)	0.5	0.125	16
	Human Serum (50%)	0.25	0.5	2.0
S. aureus PBS 864	Broth	0.25	≤0.06	0.25
	Surfactant (1%)	0.125	≤0.06	16
	Human Serum (50%)	0.25	0.5	2.0
S. aureus PBS 865	Broth	0.5	0.25	0.25
	Surfactant (1%)	0.125	≤0.06	16
	Human Serum (50%)	0.25	0.5	2.0

Table 3. Effect of surfactant or serum on the *in vitro* activity of omadacycline, doxycycline, and daptomycin against clinical isolates of *S. pneumoniae.*

Organism	Condition	MIC (mg/L)		
		OMC	DOXY	DAPTO
S. pneumoniae	Broth	0.03	0.03	0.25
ATTC 49619	Surfactant (1%)	0.03	0.03	4.0
	Human Serum (25%)	0.03	0.125	0.5
	Mouse Serum (25%)	0.06	0.125	0.25
S. pneumoniae	Broth	0.06	0.125	0.25
PBS 1339	Surfactant (1%)	0.06	0.125	16
	Human Serum (25%)	0.03	0.125	1.0
	Mouse Serum (25%)	0.06	0.125	0.5
S. pneumoniae	Broth	0.06	4.0	0.25
PBS 942	Surfactant (1%)	0.06	4.0	8.0
	Human Serum (25%)	0.03	4.0	0.5
	Mouse Serum (25%)	0.06	8.0	0.25

S. *aureus* QC Ranges:

OMC = 0.125-1.0 mg/L
Doxy = 0.125-0.5 mg/L
Dapto = 0.25-1.0mg/L

S. pneumoniae QC Ranges:

OMC = 0.015-0.125 mg/L
Doxy = 0.015-0.125 mg/L
Dapto = 0.06-0.5mg/L

Table 4. Effect of surfactant or human serum on the *in vitro* activity of omadacycline and doxycycline against clinical isolates of *E. coli.*

Organism	Condition	MIC (mg/L)	
		OMC	DOXY
E. coli ATCC 25922	Broth	0.5	0.5
	Surfactant (1%)	0.5	0.5
	Human Serum (50%)	0.25	2.0
E. coli PBS 1155	Broth	1.0	1.0
	Surfactant (1%)	0.5	0.5
	Human Serum (50%)	0.25	1.0
E. coli PBS 1167	Broth	1.0	1.0
	Surfactant (1%)	0.5	1.0
	Human Serum (50%)	0.5	2.0
E. coli PBS 1325	Broth	4.0	8.0
	Surfactant (1%)	2.0	8.0
	Human Serum (50%)	2.0	32
E. coli PBS 1215	Broth	2.0	32
	Surfactant (1%)	1.0	64
	Human Serum (50%)	1.0	>64
E. coli PBS 1154	Broth	1.0	32
	Surfactant (1%)	0.5	32
	Human Serum (50%)	0.5	>64

QC Ranges: OMC = 0.25-2.0 mg/L; Doxy = 0.12-0.5 mg/L

Table 5. Effect of surfactant or human serum on the *in vitro* activity of omadacycline and doxycycline against clinical isolates of *H. influenzae.*

Organism	Condition	MIC (mg/L)	
		OMC	DOXY
H. influenzae ATCC 49247	Broth	0.5	1.0
	Surfactant (1%)	1.0	1.0
	Human Serum (50%)	1.0	2.0
H. influenzae PBS 391	Broth	0.5	0.5
	Surfactant (1%)	0.5	0.25
	Human Serum (50%)	0.5	1.0
H. influenzae PBS 692	Broth	4.0	2.0
	Surfactant (1%)	4.0	2.0
	Human Serum (50%)	2.0	4.0
H. influenzae PBS 693	Broth	4.0	2.0
	Surfactant (1%)	2.0	2.0
	Human Serum (50%)	4.0	4.0
H. influenzae PBS 407	Broth	1.0	4.0
	Surfactant (1%)	1.0	4.0
	Human Serum (50%)	2.0	8.0
H. influenzae PBS 525	Broth	2.0	4.0
	Surfactant (1%)	2.0	4.0
	Human Serum (50%)	1.0	8.0

QC Ranges: OMC = 0.5-2.0 mg/L; Doxy = N/A

CONCLUSIONS

- Omadacycline (PTK796) *in vitro* activity was not affected by the addition of lung surfactant or serum
- These results support the potential use of omadacycline in treating pneumonia caused by susceptible bacteria

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