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The In Vitro Activity of Omadacycline and Comparators Against Anaerobic Bacteria

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ABSTRACT

Omadacycline (OMC), a broad-spectrum aminomethylcycline, has shown clinical efficacy in anaerobic acute bacterial skin and skin structure infections (ABSSSI) and in animal models of intra-abdominal anaerobic infections. Here, the in vitro activity of OMC against clinically-relevant anaerobes was similar to tigecycline, with MIC_{90} values of 1 to 8 µg/mL against *Bacteroides* spp , 0.5 µg/mL against *Clostridium difficile*, *Prevotella* spp., and *Porphyromonas asaccharolytica*, 1 µg/mL against *Peptostreptoccus* spp., and 16 µg/mL against *C. perfringens*.

CONTENTS OF NOTE

In nature, anaerobic bacteria are ubiquitous organisms of which a diverse array exists as part of the normal human microflora associated with mucous membranes[1,2]. A variety of anaerobic infections can occur, typically due to disruption of this commensal relationship with the host, and involve a comparatively less diverse group of organisms upon breach of a mucous membrane barrier at or near the site of infection. These infections are frequently polymicrobial and usually result in abscess formation[1,2]. Anaerobic infections are most often treated with β-lactams plus β-lactamase inhibitors, metronidazole (MTZ), clindamycin (CLI), carbapenems, tigecycline, and cefoxitin[1,2]. A novel aminomethylcycline, OMC, has activity against the two most common tetracycline resistance mechanisms and is currently undergoing clinical evaluation by Paratek Pharmaceuticals (Boston, MA) for the treatment of ABSSSI and community-acquired bacterial pneumonia[3]. In ABSSSI trials and in animal models of anaerobic infection (e.g. intra-abdominal infection), OMC has demonstrated efficacy against anaerobic infections[4,5].

The activity of OMC and comparators was evaluated against the following anaerobic organisms from the Micromyx repository (N = 186; Tables 1 – 2): *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, *Bacteroides ovatus*, *Clostridium difficile*, *Clostridium*

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perfringens, *Peptostreptococcus* spp., *Porphyromonas asaccharolytica*, and *Prevotella* spp. The test organisms consisted of randomly-selected, non-consecutive, non-duplicate human clinical isolates, collected from 2006 to 2016within the United States; most of the isolates were from abscesses, wounds, or infections of gall bladder, blood, or abdomen. *C. difficile* isolates were isolated from stool samples. Nine of the evaluated *P. asaccharolytica* isolates were veterinary in origin, collected in 2007 in Japan. OMC powder was provided by Paratek and was stored at -80°C. Comparator drugs included tigecycline (TGC), meropenem (MEM), moxifloxacin (MXF), CLI, MTZ, and piperacillin-tazobactam (P/T). Stock solutions of these reference compounds were prepared on each day of the assay using solvents recommended by the Clinical Laboratory Standards Institute (CLSI)[6,7]. Concentration ranges used during testing spanned relevant quality control ranges and breakpoints as established for each test compound against anaerobes[6,7]. Tazobactam was tested at a fixed concentration of 4 µg/mL in combination with piperacillin.

For *Bacteroides* spp. only, MIC determinations were made by broth microdilution; all other organisms were evaluated by agar dilution and all testing was performed in accordance with CLSI guideline M11-A8[6] and CLSI supplement M100-S26[7] using freshly-prepared *Brucella* broth and agar. Where noted, MIC values were interpreted as susceptible (S), intermediate (I), or resistant (R) in accordance with CLSI M100-S26[7], with the exception of TGC, where FDA interpretive criteria were used[8]. Relevant quality control isolates from the American Type Culture Collection (ATCC; Manassas, VA; *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, and *C. difficile* ATCC 700057) were included during testing. MIC values for QC isolates were within established quality control ranges for all drugs.

As shown in Table 1, OMC demonstrated potent activity relative to comparator agents against *Bacteroides* spp. including *B. fragilis*, *B. thetaiotaomicron*, *B. vulgatus*, and *B. ovatus*; $MIC_{50/90}$ values for OMC against these organisms were 0.5/4, 1/4, 0.12/1 and 0.5/8 µg/mL, respectively. OMC was also active against *Prevotella* spp. and *P. asaccharolytica* with $MIC_{50/90}$ values of 0.5/2 and 0.25/0.5 µg/mL, respectively (Table 1).

Against the Gram-positive anaerobes *C. difficile* and *Peptostreptococcus* spp., OMC also demonstrated potent activity by $MIC_{50/90}$, with values of 0.25/0.5 and 0.12/1 µg/mL, respectively (Table 2). However, against *C. perfringens* OMC was less active, with $MIC_{50/90}$ values of 4/16 µg/mL (Table 2).

Overall, the evaluated isolates were found to be susceptible to P/T in this study and most were susceptible to MEM and TGC (with the exception of *C. perfringens* against TGC, 40.9%S) (Tables 1 – 2). As expected, MTZ also showed good activity, with >90%S across species, except for *B. fragilis* (81%S), *B. ovatus* (80%S) and *Peptostreptococcus* spp. (77.3%S) (Tables 1 – 2). As expected, CLI and MXF had fairly poor activity in this study, with susceptibilities in the range of 38.1 to 70% for the *Bacteroides* spp. and 0 to 86.4% for the *Clostridium* spp.(Tables 1 – 2).

In conclusion, OMC had potent activity *in vitro* against Gram-negative and -positive anaerobes commonly isolated from human infections. The activity of OMC against anaerobes was similar to that reported previously[3] and also parallels that observed with TGC, an agent indicated for the treatment of anaerobes in skin and intra-abdominal infections[8], both by $MIC_{50/90}$ and MIC distribution, with values identical or within 2-fold (Tables 1 – 2). The *in vitro* activity of OMC against anaerobic pathogens along with the *in vivo* efficacy against anaerobes in animal models

of anaerobic infection and in human skin infections highlight the potential of OMC for the treatment of human anaerobic infections.

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108 Table 1. Summary of the in vitro activity (µg/mL) of OMC and comparators against anaerobes

Organism (no. of isolates)	Drug	MIC range	MIC_{50}	MIC_{90}	%S	%I	%R
Gram-negative anaerobes (*Bacteroides* spp.)							
B. fragilis (21)	OMC	0.25 - 16	0.5	4	-	-	-
	TGC	0.5 - 8	0.5	2	95.2	4.8	0.0
	MEM	0.12 - 4	0.25	1	100	0.0	0.0
	MXF	0.12 - 16	1	8	71.4	14.3	14.3
	CLI	0.06 - >32	1	>32	71.4	0.0	28.6
	MTZ	0.25 - >32	1	>32	81.0	0.0	19.0
	P/T	0.12 - 8	1	4	100	0.0	0.0
B. thetaiotaomicron (21)	OMC	0.12 - 16	1	4	-	-	-
	TGC	0.25 - 16	1	8	85.7	9.5	4.8
	MEM	0.12 - 8	0.25	2	95.2	4.8	0.0
	MXF	1 - >16	2	>16	52.3	4.8	42.9
	CLI	0.25 - >32	4	>32	38.1	19.0	42.9
	MTZ	0.25 - >32	1	2	90.5	0.0	9.5
	P/T	1 - 16	8	16	100	0.0	0.0
B. vulgatus (21)	OMC	0.06 - 2	0.12	1	-	-	-
	TGC	0.12 - 2	0.25	1	100	0.0	0.0
	MEM	0.12 - 2	0.25	0.5	100	0.0	0.0
	MXF	0.25 - >16	1	16	61.9	4.8	33.3
	CLI	≤0.03 - >32	1	>32	57.1	0.0	42.9
	MTZ	0.12 - >32	1	2	95.2	0.0	4.8
	P/T	0.25 - >16	4	8	100	0.0	0.0
B. ovatus (15)	OMC	0.06 - >16	0.5	8	-	-	-
	TGC	0.03 - >16	0.5	8	86.6	6.7	6.7
	MEM	≤0.015 - 4	0.25	2	100	0.0	0.0
	MXF	1 - >16	2	>16	53.3	6.7	40.0
	CLI	≤0.03 - >32	8	>32	40.0	6.7	53.3
	MTZ	0.12 - >32	1	>32	80.0	0.0	20.0
	P/T	≤0.015 - 16	4	8	100	0.0	0.0
Gram-negative bacilli (non-*Bacteroides* spp.)							
Prevotella spp. (22)	OMC	0.12 - 8	0.5	2	-	-	-
	TGC	0.06 - 16	1	4	95.5	0.0	4.5
	MEM	0.03 - 1	0.12	0.5	100	0.0	0.0
	MXF	0.5 - >16	1	>16	63.6	22.8	13.6
	CLI	0.06 - >32	2	>32	51.0	4.5	44.5
	MTZ	0.25 - >32	1	8	95.5	0.0	4.5
	P/T	≤0.06 - 32	≤0.06	4	100	0.0	0.0

Organism	Drug	MIC range	MIC_{50}	MIC_{90}	%S	%I	%R
P. asaccharolytica (21)	OMC	0.06 - 2	0.25	0.5	-	-	-
	TGC	0.03 - 1	0.25	0.5	100	0.0	0.0
	MEM	≤0.015 - 0.25	0.03	0.12	100	0.0	0.0
	MXF	0.12 - >16	0.25	16	85.7	0.0	14.3
	CLI	≤0.03 - >32	0.5	>32	80.9	4.8	14.3
	MTZ	0.06 - >32	0.5	2	90.5	0.0	9.5
	P/T	≤0.06 - 0.5	≤0.06	0.25	100	0.0	0.0

MIC_{50} = MIC value at which inhibition of at least 50% of evaluated isolates was observed; MIC_{90} = MIC value at which inhibition of at least 90% of evaluated isolates was observed; P/T = piperacillin-tazobactam (tazobactam tested at a constant concentration of 4 µg/mL; piperacillin MIC shown in tables); %S = percent susceptible; %I = percent intermediate; %R = percent resistant

MIC values interpreted based on CLSI breakpoints (6) excluding tigecycline where MIC values were interpreted based on FDA prescribing information for Tygacil® (7)

116 Table 2. Activity (µg/mL) of OMC and comparators against Gram-positive anaerobes

Organism (no. of isolates)	Drug	MIC range	MIC_{50}	MIC_{90}	%S	%I	%R
C. difficile (21)	OMC	0.25 - 8	0.25	0.5	-	-	-
	TGC	0.25 - 4	0.25	0.25	100	0.0	0.0
	MEM	0.5 - 4	2	2	100	0.0	0.0
	MXF	1 - >16	2	>16	61.9	0.0	38.1
	CLI	4 - >32	8	>32	0.0	38.1	61.9
	MTZ	0.25 - 8	0.5	1	100	0.0	0.0
	P/T	4 - 16	8	16	100	0.0	0.0
C. perfringens (22)	OMC	0.12 - 16	4	16	-	-	-
	TGC	0.25 - >16	8	>16	40.9	9.1	50.0
	MEM	≤0.015 - 8	0.015	1	95.5	4.5	0.0
	MXF	0.5 - >16	0.5	4	86.4	4.5	9.1
	CLI	0.06 - >32	2	>32	72.8	4.5	22.7
	MTZ	0.5 - >32	1	4	90.9	0.0	9.1
	P/T	≤0.06 - 32	0.5	16	100	0.0	0.0
Peptostreptococcus spp.[1] (22)	OMC	0.06 - 2	0.12	1	-	-	-
	TGC	0.06 - 4	0.12	2	100	0.0	0.0
	MEM	≤0.015- 16	0.25	0.5	95.5	0.0	4.5
	MXF	0.25 - >16	0.5	8	77.2	0.0	22.8
	CLI	0.06 - >32	0.5	>32	63.7	4.5	31.8
	MTZ	0.12 - >32	0.5	>32	77.3	0.0	22.7
	P/T	≤0.06 - 32	0.25	2	100	0.0	0.0

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118 MIC_{50} = MIC value at which inhibition of at least 50% of evaluated isolates was observed; MIC_{90} = MIC value at which inhibition of at least
119 90% of evaluated isolates was observed; P/T = piperacillin-tazobactam (tazobactam tested at a constant concentration of 4 µg/mL; piperacillin
120 MIC shown in tables); %S = percent susceptible; %I = percent intermediate; %R = percent resistant

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122 MIC values interpreted based on CLSI breakpoints (6) excluding tigecycline where MIC values were interpreted based on FDA prescribing
123 information for Tygacil® (7)

124 [1] *Peptostreptococcus* spp. include 11 *P. micros* and 11 *P. anaerobius*

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