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Susceptibility Testing Characteristics and Quality Control Guidelines for Omiganan Pentahydrochloride, A Topical Cationic Peptide

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ABSTRACT

Background: Omiganan (OMI) pentahydrochloride, formerly MBI226, is a 12 amino acid cationic peptide with documented broad-spectrum activity against Gram-positive and -negative bacteria and some fungi (MIC, ≤ 64 µg/ml) and is in clinical development for the prevention of catheter related infections. Standardization of in vitro OMI testing is needed for clinical and regulatory purposes.
Methods: Parameters of OMI susceptibility testing were studied in 2 Phases: 1) MICs of NCCLS quality control (QC) strains were compared in cation-adjusted (CA) and -unadjusted (UA) Mueller-Hinton broth (MHB), in freshly-made and frozen trays (-70ºC), and OMI stability studied in frozen storage x 45 d; and 2) QC trials (7 sites) by NCCLS M23-A2 guidelines with OMI diluted in CA- or UA-MHB (3 lots each), and MBC tests of selected species (10). RPMI-1640 + MOPS (3 lots) was used to test 2 yeast QC strains.
Results: Replicate tests of QC strains in fresh and frozen reference trays produced near equal results, but a trend toward lower MIC values was noted in UA-MHB. OMI MIC ranges by QC strain were (CA/UA-MHB MIC in µg/ml): *S. aureus* 29213 (8-32/same), *E. faecalis* 29212 (32-128/same), *E. coli* 25922 (16-64/same), *P. aeruginosa* 27853 (64-256/16-64) and *S. pneumoniae* 49619 (32-128/16-64). The OMI MIC ranges established by the QC trial were (CA/UA-MHB MIC): *S. aureus* (8-64/4-32), *E. faecalis* (32-128/same), *S. pneumoniae* (32-128/16-64), *E. coli* (16-64/8-32), *P. aeruginosa* (64-256/8-64), *C. krusei* 6258 (16-128) and *C. parapsilosis* 22019 (32-128). OMI MBC results approximated the MIC or were no greater than 2X higher.
Conclusions: OMI was observed to be a microbicidal cationic peptide with MIC results slightly influenced (increased by cationic binding) by CA-MHB. This effect was greatest with *P. aeruginosa* (4X) and QC ranges for OMI susceptibility tests using NCCLS MIC methods were established for 2 MH media to monitor its long-term clinical efficacy.

INTRODUCTION

Omiganan pentahydrochloride (formerly MBI 226) is a novel, topical cationic peptide (sequence: ILRWPWWPWRRK-amide) analog of indolicidin that was originally purified from the cytoplasmic granules of bovine neutrophils. Omiganan pentahydrochloride has demonstrated in vitro activity against a wide variety of microorganisms including Gram-positive and -negative bacteria and fungi, and could be used in venous catheter care. This compound is rapidly bactericidal and interacts with the cytoplasmic membranes of both Gram-positive and -negative bacteria. In *Staphylococcus aureus*, omiganan pentahydrochloride acts by depolarizing the cytoplasmic membrane resulting in cell disruption and death. This compound also shows a dose-dependent inhibitory effect on whole cell protein, RNA, and DNA synthesis in *S. aureus*. Exposure to omiganan pentahydrochloride resulted in *Escherichia coli* outer membrane permeabilization. A topical 1% gel preparation of omiganan pentahydrochloride is currently in Phase III development for the prevention of catheter-related infections.

In the United States, > 200,000 cases of blood stream infections (BSIs) occur annually, and skin flora are the primary source of catheter-related BSI. The Gram-positive cocci, including *S. aureus*, coagulase-negative staphylococci (CoNS), enterococci and streptococci accounted for > 53% of bacterial BSI evaluated by the SENTRY Program. In addition, data from the National Nosocomial Infection Surveillance Study (NNISS) and other multicenter studies have shown that *Candida* is a leading cause of BSI, especially in the intensive care units. Catheter-related BSIs (CRBSI) represent a significant problem in health care settings. Infectious complications occur in 5 - 19% of patients receiving a central venous catheter and efforts have been made to minimize these infections. The purpose of this study was to evaluate the in vitro antimicrobial activity of omiganan pentahydrochloride tested against recent clinical isolates of bacteria and yeast that could cause CRBSI.

MATERIALS AND METHODS

Organisms tested: All isolates were collected from clinical samples in North America and stored as stock cultures at –70°C in TSB or defibrinated rabbit blood for bacteria or in sterile distilled water at room temperature for yeast and *P. aeruginosa*. Some of these isolates were obtained from the omiganan pentahydrochloride clinical trial.

Antimicrobial susceptibility testing: Susceptibility testing of all isolates was performed utilizing NCCLS reference broth microdilution methods. Omiganan pentahydrochloride was provided by Micrologix Biotech Inc. (Vancouver, Canada). Comparator agents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) or obtained from their respective manufacturers in the USA. The bacterial isolates were tested in cation-adjusted (CA)- and unadjusted (UA)-Mueller-Hinton (MH) broth. The panels were incubated at 35ºC in an ambient air environment for 16-18 hours for Gram-negative bacilli, and 20-24 hours for the Gram-positive and fastidious organisms. Panels were read manually and an endpoint of no visible growth was established as the minimum inhibitory concentration (MIC) per NCCLS criteria. An omiganan pentahydrochloride susceptible breakpoint of ≤ 256 µg/ml was conservatively selected based on one-fortieth the concentration of the compound in the topical gel (10,000 µg/ml), and this breakpoint was used for comparison purposes only. MIC values for comparator antimicrobial agents were interpreted using NCCLS M100-S14 guidelines unless noted. Concurrent QC was performed by testing control strains as follows: *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 29213, and *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

MATERIALS AND METHODS (Continued)

For the yeast isolates, a suspension equal to a 0.5 McFarland standard was made, diluted 1:500 in RPMI 1640 broth with MOPs buffer and inoculated into the thawed panels to a final concentration of 0.5 – 2.5 x 10^3 CFU/ml. Panels were incubated in an ambient air environment at 35ºC and were read at both 24 and 48 hours of growth. Quality control was performed by testing ATCC strains: *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258.

All frozen (-70ºC) panels representing the three media types were included in a 120-day stability study. Panels were tested in triplicate at day 0, 7, 14, 21, 28, 45, 60, 90, and 120 post manufacture for each of five bacterial and two yeast QC strains.

QC trials and pre-QC pilots were performed according to NCCLS M23-*A2* (2001). Collaborating laboratories were: JMI Laboratories (Iowa), TREK Diagnostic Systems, Inc. (Ohio), University of Alberta (Edmonton, AB), The Cleveland Clinic Foundation (Ohio), University of Texas Medical Center (Houston, Texas), University of Rochester (New York), and University of Washington (Washington).

Bactericidal activity: Ten strains, including *Klebsiella pneumoniae*, *E. coli* (ATCC 25922), *Acinetobacter* spp., *P. aeruginosa* (ATCC 27853), two *S. aureus* (one oxacillin-resistant, one oxacillin-susceptible), *S. epidermidis*, two *Enterococcus* spp. (one wild type and one vancomycin-resistant), and *C. albicans* were tested by the kill-curve methodology to evaluate the bactericidal activity of omiganan pentahydrochloride. Bacterial kill curves were performed in CA-MH broth and *C. albicans* kill curves were performed in RPMI-1640 with MOPs buffer. Omiganan pentahydrochloride activity was tested at timed intervals of T0, T0.5, T2, T6, and T24 hours at 1X, 2X, 4X, and 8X the MIC. Minimal bactericidal and fungicidal concentrations (MBC and MFC, respectively) were assessed by plating the broth from the MIC well and from those three $\log_2$ dilutions above the MIC for each organism onto appropriate growth media. Quantitative colony counts were done on the starting inoculum at the time the MIC test was performed. The lowest concentration of antimicrobial agent that kills ≥ 99.9% of the starting test inoculum was defined as the cidal endpoint. A total of eight strains, including *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* 24-1920A, *C. albicans* 15-10082A, and *C. albicans* 13-13547A were selected for this experiment.

RESULTS

- Omiganan pentahydrochloride has significant activity against Gram-positive cocci (MIC_{90}s, 4 - 256 µg/ml), *Bacillus* spp. (MIC_{90}, 32 µg/ml), *Corynebacterium* spp. (MIC_{90}, 8 µg/ml), Enterobacteriaceae (MIC_{90}, 128 µg/ml), *P. aeruginosa* (MIC_{90}, 256 µg/ml) and yeast (MIC_{50}, 256 µg/ml), see Table 1.
- Omiganan pentahydrochloride was more active in cation-unadjusted MH broth (two- to four-fold lower MIC values), especially versus *P. aeruginosa* (Table 2).
- Bactericidal or fungicidal activity was demonstrated (Table 3) at concentrations equal to or two-fold greater than the MIC results. These results were confirmed by kill-curve analyses (Table 4) where concentration-dependent cidal action was noted, even when tested against strains resistant to potent parenteral agents (example: vancomycin-resistant *E. faecium*; Figure 1).
- Omiganan pentahydrochloride was observed to be stable at -70ºC frozen storage in broth microdilution trays (CA or UA-MHB or RPMI-1640 broth) for 120 days, see Table 5.
- QC ranges for MIC tests in three media were established by Tier I and II protocols, each validating the findings of the other (Table 6). The disk diffusion test could not be optimized, data not shown.

Figure 1: Time kill-curve for omiganan pentahydrochloride tested against a vancomycin-resistant (*vanA*) *E. faecium*.

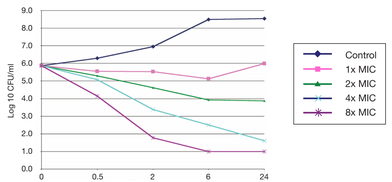

Table 1. Summary of the activity of omiganan pentahydrochloride tested in cation-adjusted Mueller-Hinton broth (modified from Sader et al., in press; 1,651 isolates).

	Cum. % inhibited at MIC (µg/ml):									
Organism (no. tested)	≤1	2	4	8	16	32	64	128	256	512
Staphylococci										
S. aureus (199)	0.0	0.5	1.0	22.1	95.5	99.0	100.0	-	-	-
CoNS (218)	13.3	33.0	91.3	99.5	100.0	-	-	-	-	-
Enterococci										
E. faecalis (100)	0.0	0.0	0.0	0.0	2.0	6.0	56.0	100.0	-	-
E. faecium (101)	0.0	2.0	14.9	80.2	100.0	-	-	-	-	-
Streptococci (202)	0.0	0.5	2.0	5.9	39.6	59.4	80.2	89.1	99.5	100.0
Bacillus spp. (103)	17.5	29.1	34.0	37.9	71.8	94.2	100.0	-	-	-
Corynebacterium spp. (103)	17.5	44.7	79.6	96.1	99.0	99.0	100.0	-	-	-
Enterobacteriaceae (309)	0.0	0.0	0.0	4.5	48.5	79.0	86.4	93.2	96.1	99.7[a]
P. aeruginosa (102)	0.0	0.0	0.0	0.0	2.0	3.9	29.4	88.2	100.0	-
Candida spp. (214)	0.0	0.0	0.0	1.9	10.3	23.4	71.0	80.4	96.7	99.5

a. Only one strain (*E. cloacae*) at > 512 µg/ml.

Table 2. Antimicrobial activity of omiganan pentahydrochloride (MBI226) and two agents tested against *P. aeruginosa* (n=102).

	MIC (µg/ml)			% by category	
Antimicrobial agent	50%	90%	Range	Susceptible	Resistant
MBI 226 (UA-MH)[a]	32	64	8-64	(100.0)[b]	-[c]
MBI 226 (CA-MH)[d]	128	256	16-256	(100.0)[b]	-
Ciprofloxacin	≤0.25	>2	≤0.25->2	78.4	19.6
Gentamicin	2	>8	≤1->8	86.3	10.8

a. Cation-unadjusted Mueller-Hinton broth.
b. A susceptible breakpoint of ≤ 256 µg/ml was used for comparison purposes.
c. - = no breakpoint has been established by the NCCLS.
d. Cation-adjusted Mueller-Hinton broth.

Table 3. MIC and MBC (MFC)[a] comparisons for six bacteria and two *C. albicans* isolates tested in several Mueller-Hinton broth or RPMI mediums.

	MIC/MBC or MFC in µg/ml by medium		
Organism	Cation-adjusted MHB	Cation-unadjusted MHB	RPMI
E. coli ATCC 25922	32/128	16/32	-
E. faecalis ATCC 29212	64/128	64/128	-
P. aeruginosa ATCC 27853	64/128	16/16	-
S. aureus ATCC 29213	8/8	8/8	-
S. aureus MRSA 24-1920A	16/16	8/8	-
S. pneumoniae ATCC 49619	32/64	32/32	-
C. albicans 15-10082A			
RPMI Lot #1	-	-	64/128[b]
RPMI Lot #2	-	-	64/64[b]
C. albicans 13-13547A			
RPMI Lot #1	-	-	64/128[b]
RPMI Lot #2	-	-	64/128[b]

a. MFC = minimum fungicidal concentration.
b. All yeast MIC results were read at 24 hours of incubation.

Table 4. Kill curve kinetic studies in cation-unadjusted Mueller-Hinton broth for 10 selected organisms using four concentrations of omiganan pentahydrochloride and monitoring at 0.5, 2, 6, and 24 hours.

		CFU/ml at T (hrs):					
Organisms	Concentration related to MIC	0	0.5	2	6	24	MIC (µg/ml)
S. aureus ATCC 29213							
	Control	4.8E6	5.7E6	3.3E7	5.2E8	8.5E8	8
	MIC	-	2.0E6	1.3E5	1.6E4	3.2E8	-
	2X MIC	-	1.2E6	3.8E4	1.6E3[a]	6.2E5	-
	4X MIC	-	3.8E5	7.8E3	1.3E2[a]	1.1E4	-
	8X MIC	-	1.5E5	1.1E3[a]	1.0E2[a]	1[a]	-
S. aureus MBI105[b]							
	Control	2.8E6	4.8E6	2.0E7	3.7E8	7.1E8	8
	MIC	-	1.3E5	1.5E4	5.9E4	2.3E7	-
	2X MIC	-	1.8E4	1.7E3[a]	7.7E6	6.1E6	-
	4X MIC	-	1.2E4	2.3E2[a]	1.7E5	4.9E5	-
	8X MIC	-	7.5E3	2.9E2[a]	1[a]	6.0E2[a]	-
S. epidermidis 6-313A[b]							
	Control	1.8E5	1.3E5	1.7E5	4.7E6	1.3E8	2
	MIC	-	3.6E4	2.0E3	2.0E4	5.8E4	-
	2X MIC	-	1.7E4	8.5E2	1.0E1[a]	1.0E5	-
	4X MIC	-	4.7E3	1.6E2[a]	1.0E1[a]	8.0E4	-
	8X MIC	-	1.2E3	1.0E1[a]	1.0E1[a]	1.0E3	-
E. faecium 27-308A (VSE)							
	Control	2.1E6	2.1E6	2.2E7	3.2E8	6.0E8	8
	MIC	-	9.5E5	2.0E5	1.7E4	3.3E5	-
	2X MIC	-	3.6E5	3.2E4	1.4E3[a]	3.1E2[a]	-
	4X MIC	-	4.4E4	2.7E3	1.3E2[a]	6.0E1[a]	-
	8X MIC	-	7.4E3	2.6E3	5.0E1[a]	1[a]	-
E. faecium 15-206A[c]							
	Control	7.3E5	2.0E6	9.1E6	3.1E8	3.5E8	8
	MIC	-	3.5E5	3.4E5	1.4E5	9.8E5	-
	2X MIC	-	2.0E5	4.2E4	8.5E3	7.6E3	-
	4X MIC	-	1.2E5	2.5E3	3.2E2[a]	4.0E1[a]	-
	8X MIC	-	1.4E4	6.0E1[a]	1.0E1[a]	1.0E1[a]	-
E. coli ATCC 25922							
	Control	1.4E6	2.8E6	4.0E7	5.0E8	8.7E8	16
	MIC	-	1.7E6	1.4E6	1.7E6	1.9E8	-
	2X MIC	-	1.5E6	6.0E5	3.8E4	2.9E8	-
	4X MIC	-	2.2E5	5.4E4	3.4E4	5.7E2[a]	-
	8X MIC	-	9.0E2[a]	2.3E2[a]	1[a]	1[a]	-
K. pneumoniae 21-1940A							
	Control	9.0E5	4.3E6	1.1E8	4.4E8	6.7E8	16
	MIC	-	3.5E6	1.3E7	2.3E8	7.5E8	-
	2X MIC	-	2.1E6	4.9E5	8.2E4	3.0E8	-
	4X MIC	-	2.7E5	2.3E4	1.1E4	7.3E3	-
	8X MIC	-	2.7E4	6.5E1[a]	1[a]	1[a]	-
A. baumannii 101-2823A							
	Control	3.2E6	4.4E6	2.7E7	7.5E7	2.2E9	8
	MIC	-	6.7E6	1.4E7	2.0E7	1.3E9	-
	2X MIC	-	4.2E6	1.6E5	7.2E4	8.2E5	-
	4X MIC	-	1.2E6	6.6E2[a]	1[a]	1[a]	-
	8X MIC	-	4.1E2[a]	1[a]	1[a]	1.0E1[a]	-
P. aeruginosa ATCC 27853							
	Control	2.8E6	2.3E6	2.6E7	2.2E8	3.0E9	16
	MIC	-	3.4E6	3.2E6	1.6E8	7.5E8	-
	2X MIC	-	3.4E6	1.9E7	9.1E7	1.8E9	-
	4X MIC	-	2.5E5[a]	2.2E5[a]	1.0E5	2.1E8	-
	8X MIC	-	1.0E2[a]	4.3E2[a]	4.3E4	1.2E6	-
C. albicans 15-10082A							
	Control	3.0E6	2.5E6	3.5E6	8.9E6	2.3E7	64
	MIC	-	2.0E6	1.9E6	3.8E6	9.3E6	-
	2X MIC	-	1.9E6	2.4E5	5.6E3	3.0E4	-
	4X MIC	-	3.5E5	9.1E2[a]	1[a]	1[a]	-
	8X MIC	-	9.3E3	2.0E1[a]	1[a]	1[a]	-

a. Indicates bactericidal results (≥ 3 $\log_{10}$ killing), usually occurring at 2 to 6 hours.
b. Oxacillin-resistant strains.
c. Vancomycin-resistant strains.

Table 5. Stability of omiganan pentahydrochloride as measured by selected QC strain MIC results after frozen storage intervals ranging from 0 to 120 days (CA-MHB or RPMI-1640 media).

	MIC (µg/ml) at:		
QC strain	Baseline	Last day (120)	Distribution overall
E. coli ATCC 25922	32(3)[a]	32(3)	16(8), 32(16)
E. faecalis ATCC 29212	64(3)	64(3)	32(1), 64(23)
P. aeruginosa ATCC 27853	64(3)	64(3)	64(20), 128(4)
S. aureus ATCC 29213	16(3)	8(2), 16(1)	8(15), 16(9)
S. pneumoniae ATCC 49619	64(3)	32(1), 64(2)	32(14), 64(10)
C. krusei ATCC 6258[b]	16(6)	16(4), 32(2)	16(33), 32(3)
C. parapsilosis ATCC 22019[b]	16(6)	16(1), 32(5)	16(8), 32(27), 64(1)

a. Occurrences are listed in parenthesis.
b. Tested in two media lots (RPMI-1640), read at 24 hours.
c. All results were interpreted as indicating stability for 120 days stored at -70ºC.

Table 6. Bacterial and yeast QC results from Tier 1 pilot and multi-center trials.[a]

	Omiganan MIC (µg/ml)	
	Proposed range by medium type	% in range by medium type
QC organism	Adjusted/unadjusted	Adjusted/unadjusted[b]
S. aureus ATCC 29213	8-64(8-32)/4-32(8-32)[b]	99.5/100.0
E. faecalis ATCC 29212	32-128(32-128)/32-128(32-128)	100.0/100.0
S. pneumoniae ATCC 49619	32-128(32-128)/16-64(16-64)	100.0/100.0
E. coli ATCC 25922	16-64(16-64)/8-32(16-64)	99.0/100.0
P. aeruginosa ATCC 27853	64-256(64-256)/8-64(16-64)	100.0/100.0
C. krusei ATCC 6258	16-128(8-32)/-	100.0/-
C. parapsilosis ATCC 22019	32-128(16-64)/-	99.0/-

a. Modified from Anderegg et al. (2004).
b. Range in parenthesis was derived from the Phase I pilot study (University of Alberta).

CONCLUSIONS

- Omiganan pentahydrochloride appears to be an excellent bactericidal/fungicidal topical cationic-peptide for use in minimizing catheter-related colonization and/or infections.
- This agent will be used as a 1% gel preparation having a wide spectrum of activity against pathogenic bacteria and yeast.
- In vitro stability and susceptibility testing parameters have been established for diagnostic use in clinical microbiology laboratories.

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