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In Vitro Spectrum of Pexiganan Against Pathogens from Diabetic Foot Infections and with Selected Resistance Mechanisms

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ABSTRACT

Background: Pexiganan (PEX) is a 22-amino acid synthetic analogue of peptide magainin II that is currently in Phase 3 clinical trials as a topical antimicrobial (PEX cream 0.8% [8,000 µg/mL pexiganan free base]) for treatment of mild infections of diabetic foot ulcer (DFI).

Methods: Against PEX and comparators we tested bacterial isolates from the 2013 SENTRY Antimicrobial Surveillance Program designated as pathogens from DFI (n=46) and Gram-negative (GN) and -positive (GP) pathogens (n=63) from various infection types that harbored selected resistance (R) mechanisms/ phenotypes. Using CLSI reference methods, we performed broth microdilution MIC testing in cation-adjusted Mueller-Hinton broth.

Results: The MIC_{50} and MIC_{90} against all organisms tested from DFI were 16 and 32 µg/mL, respectively. *E. coli, K. pneumoniae, C. koseri, E. cloacae, Acinetobacte*r spp. and *P. aeruginosa* MIC values ranged from 8-16 µg/mL. Two *M. morganii,* 1 *P. vulgaris* and 1 *S. marcescens* exhibited MIC values >32 µg/mL. PEX MIC values among *S. aureus* (8 MRSA and 12 MSSA), β-hemolytic streptococci, and *E. faecium* ranged from 8-32 µg/mL. When tested against a select group of microorganisms with known R phenotypes/genotypes, PEX activity was not adversely affected for Enterobacteriaceae that produced ESBL, plasmidic AmpC, KPC or NDM-1 enzymes. MBL production or resistance to other commonly used antimicrobials did not adversely influence PEX activity against *P. aeruginosa* or *Acinetobacter* spp. PEX was active against MRSA clones frequently isolated from both community and hospital-associated infections in the USA. Decreased susceptibility to vancomycin did not affect PEX activity against *S. aureus*. R to vancomycin did not adversely affect PEX potency against *E. faecium*. *E. faecalis* appears to be intrinsically less susceptible to PEX (MIC, 32-256 µg/mL).

Conclusions: PEX, a peptide with a novel mechanism of action compared to conventional agents, demonstrates potent, broad-spectrum activity against contemporary GN and GP from DFI and selected antibiotic-resistant bacteria. The "all organism" MIC_{90} of 32 µg/mL for PEX in this study was >250-fold below the concentration of PEX in the cream/delivery vehicle. PEX should be further studied in infected patients with DFI as well as other wound/skin infections for whom topical therapy is appropriate.

INTRODUCTION

Pexiganan is a 22-amino acid synthetic analogue of peptide magainin II in late stage development as a topical agent (pexiganan cream 0.8% [8,000 µg/mL pexiganan free base]) for treatment of mild infections of diabetic foot ulcers. Magainins are broad-spectrum cationic peptides that selectively damage bacterial membranes through mechanisms that make development of resistance extremely difficult. In diabetic foot infections (DFI), *Staphylococcus aureus* and *Streptococcus* species are the most common pathogens in mild infections (that are usually treated with oral antibiotics) and *S. aureus*, *Streptococcus* spp., Enterobacteriaceae and obligate anaerobes are associated with moderate or severe infections.

The available susceptibility profiles for pexiganan were published in the late 1990s by Ge et al. We therefore performed this study to determine if any changes in susceptibility to pexiganan have emerged over the past two decades, especially to pathogens with newer types of resistance.

MATERIALS AND METHODS

Organisms: We selected 46 bacterial isolates from the 2013 Global SENTRY Surveillance Program that were designated by the investigational site as pathogens in DFI: Enterobacteriaceae (15; includes *Escherichia coli* [6]; *Enterobacter cloacae* [2]; *Citrobacter* spp. [1]; *Proteus vulgaris* [1]; *Morganella morganii* [2]; *Klebsiella pneumoniae* [2]; *Serratia marcescens* [1]), *P. aeruginosa* (6), *Acinetobacter baumannii* (1; resistant to ≥4 antimicrobial classes); *Streptococcus agalactiae* (2); *Streptococcus pyogenes* (1); *Enterococcus faecium* (1); and *S. aureus* (20; includes methicillin-resistant [MRSA] and -susceptible [MSSA]). We selected an additional collection of 63 Gram-positive and -negative isolates from various infection types and harboring selected resistance mechanisms and phenotypes. Resistance genotypes/phenotypes included: *S. aureus* (two VRSA, one hVISA, one VISA, four community-acquired *S. aureus* [USA 300], and two hospital acquired *S. aureus* [USA200]); Enterococci (*E. faecalis* [2 VanA and 2 VanB isolates], *E. faecium* [3 VanA and 3 VanB isolates]); Enterobacteriaceae (CTX-M-2, -14, -15; DHA-1, -2; CMY-2, -6; FOX-5; SHV-12, -27, -31; OXA-30; KPC-2, -3; NDM-1 and TEM-10 producing isolates); *P. aeruginosa* (two carbapenem resistant and one IMP-1 and VIM-2 containing isolate); and *A. baumannii* (two MDR [resistant to ≥4 antimicrobial classes]).

Susceptibility testing: Broth microdilution MIC testing was performed according to Clinical and Laboratory Standards Institute (CLSI) standard methods (document M07-A9 [2012]) using MIC panels produced by JMI Laboratories. Media were cation-adjusted Mueller-Hinton broth (CA-MHB; Ca++ at 20-25 mg/L; Mg++ at 10-12.5 mg/L) supplemented with 2.5-5% lysed horse blood for streptococci testing. Interpretive criteria for comparator antimicrobials were those as published by CLSI (M100-S24; 2014). Quality control was performed per CLSI M07-A9 [2012] and CLSI M100-S24 [2014] recommendations and guidelines using the following strains: *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *Streptococcus pneumoniae* ATCC 49619.

RESULTS

- The activity of pexiganan against the two sets of isolates are presented in **Tables 1** and **2**.
- The MIC_{50} and MIC_{90} against all DFI organisms tested were 16 and 32 µg/mL, respectively. There were only four isolates (8.7%) with MIC values >32 µg/mL (actual MIC, >512 µg/mL): three indole-positive *Proteae* (two *M. morganii,* one *P. vulgaris*) and one *S. marcescens* (**Table 1**).
- Pexiganan MIC values for 73.3% of the DFI Enterobacteriaceae isolates ranged from 8 to16 µg/mL (**Table 1**). This included E*. coli* (6), *K. pneumoniae* (2), *C. koseri* (1)*,* and E. *cloacae* (2; see **Table 1**). Only indole-positive *Proteae* and *S. marcescens* exhibited elevated MIC values to pexiganan. Two of the four isolates with elevated pexiganan MIC values were also resistant to levofloxacin.
- The DFI *Acinetobacter* spp. and *P. aeruginosa* pexiganan MIC values were 8-16 µg/mL, which was similar to the MIC value obtained with the *P. aeruginosa* ATCC 27853 QC strain (**Table 3**).
- Among *S. aureus* isolates from DFI (8 MRSA and 12 MSSA), the pexiganan MIC values were either 16 or 32 µg/mL (**Table 1**). Pexiganan activity did not vary based on methicillin-susceptibility status.
- Pexiganan was highly active against β-hemolytic streptococci from DFI (two *S. agalactiae* and one *S. pyogenes)*, MIC range 8-16 µg/mL. One *E. faecium* strain was susceptible to pexiganan with a MIC value of 8 µg/mL (**Table 1**).
- In the *E. coli* strains in the select group of resistant phenotype/genotypes, those strains producing ESBL, plasmidic AmpC or NDM-1 β-lactamases were very susceptible to pexiganan with MIC values of either 8 or 16 µg/mL, which were similar to those obtained with *E. coli* ATCC 25922 (**Tables 2**, **3**).
- Pexiganan was also active against *Klebsiella* spp. strains with various β-lactamases types, including ESBLs, plasmidic AmpC, KPC-types and NDM-1 (**Table 2**). Pexiganan MIC values were 4 to 32 µg/mL, except for two strains with pexiganan MIC values of 128 (a KPC-2 producing strain) and >256 µg/mL (a SHV-12 producing strain; **Table 2**). These two strains also exhibited decreased susceptibility to colistin and polymyxin B. MIC values for colistin and polymyxin B were 2 µg/mL for the KPC-producing strain and >4 µg/mL with the SHV-12 producing strain (data not shown).
- Two MDR *Acinetobacter* spp. and four *P. aeruginosa* strains, including IMP-1 and VIM-2 producing strains, exhibited pexiganan MIC values of 8 µg/mL (**Table 2**).
- Among *S. aureus*, CA-MRSA USA300, HA-MRSA USA100, hVISA and VRSA strains had pexiganan MIC values of either 8 or 16 µg/mL, while the VISA strain showed a pexiganan MIC of 32 µg/mL (**Table 2**).
- Vancomycin-resistant *E. faecium* strains were very susceptible to pexiganan with MIC values of either 4 or 8 µg/mL. Vancomycin-resistant *E. faecalis* strains, however, showed higher pexiganan MIC values (64 to >256 µg/mL; **Table 2**).

Table 1. MIC distribution for pexiganan when tested against pathogens causing diabetic foot infections selected from the SENTRY Antimicrobial Surveillance Program in 2013.

Organism group (No. tested)	No. of strains at MIC (µg/mL; cumulative %): ≤4	8	16	32	64	128	256	512	>512	MIC_{50}	MIC_{90}
S. aureus (20)	--	--	15 (75.0)	5 (100.0)						16	32
MSSA (12)	--	--	8 (66.7)	4 (100.0)						16	32
MRSA (8)	--	--	7 (87.5)	1 (100.0)						16	--
BHS (3)[a]	--	2 (66.7)	1 (100.0)							8	--
E. faecium (1)	--	1 (100.0)								8	--
Enterobacteriaceae (15)	--	10 (66.7)	1 (73.3)	0 (73.3)	0 (73.3)	0 (73.3)	0 (73.3)	0 (73.3)	4 (100.0)	8	>512
E. coli (6)	--	6 (100.0)								8	--
K. pneumoniae (2)	--	2 (100.0)								8	--
IPP (3)[b]	--	--	--	--	--	--	--	--	3 (100.0)	>512	--
Other (4)[c]	--	2 (50.0)	1 (75.0)	0 (75.0)	0 (75.0)	0 (75.0)	0 (75.0)	0 (75.0)	1 (100.0)	8	--
P. aeruginosa (6)	--	4 (66.7)	2 (100.0)							8	--
A. baumannii (1)	--	1(100.0)								8	--
All organisms (46)										16	32

a. BHS = β-hemolytic streptococci, includes *S. agalactiae* (2), *S. pyogenes* (1).
b. IPP = Indole-positive Proteae, includes *M. morganii* (2), *P. vulgaris* (1).
c. Other includes *C. koseri* (1), *E. cloacae* (2), *S. marcescens* (1).

Table 2. MIC distribution for pexiganan when tested against pathogens with selected phenotypes and genotypes.

Organism group (No. tested)	No. of strains at MIC (µg/mL; cumulative %): ≤4	8	16	32	64	128	256	>256	MIC_{50}	MIC_{90}
S. aureus (10)[a]	--	2 (20.0)	7 (90.0)	1 (100.0)					16	16
E. faecalis (4)[b]	--	--	--	--	1 (25.0)	0 (25.0)	1 (50.0)	2 (100.0)	256	--
E. faecium (6)[c]	4 (80.0)	2 (100.0							≤4	--
Enterobacteriaceae (37)[d]	1 (2.7)	24 (67.6)	9 (91.9)	1 (94.6)	0 (94.6)	1 (97.3)	0 (97.3)	1 (100.0)	8	16
E. coli (13)	--	11 (84.6)	2 (100.0)						8	16
K. pneumoniae (15)	--	6 (40.0)	6 (80.0)	1 (86.7)	0 (86.7)	1 (93.3)	0 (93.3)	1 (100.0)	16	128
K. oxytoca (4)	1 (25.0)	2 (75.0)	1 (100.0)						8	--
E. cloacae (5)	--	5 (100.0)							8	--
P. aeruginosa (4)[e]	--	3 (75.0)	1 (100.0)						8	--
A. baumannii (2)[f]	--	2 (100.0)							8	--

a. Includes 2 VRSA, 1 hVISA, 1 VISA, 4 community-acquired SA (USA 300), and 2 hospital acquired *S. aureus* (USA200).
b. Includes 2 VanA and 2 VanB isolates.
c. Includes 3 VanA and 3 VanB isolates.
d. Includes CTX-M-2, -14, -15; DHA-1, -2; CMY-2, -6; FOX-5; SHV-12, -27, -31; OXA-30; KPC-2, -3; NDM-1 and TEM-10 producing isolates.
e. 2 carbapenem resistant, 1 IMP-1 and 1 VIM-2 containing isolate.
f. 2 MDR isolates (resistant to ≥4 antimicrobial classes).

Table 3. MIC results obtained from testing quality control strains with pexiganan.

Organism (no. of tests)	No. of occurrences at MIC (µg/mL) of: 8	16	32	64	Organism (no. of tests)	No. of occurrences at MIC (µg/mL) of: 8	16	32	64
S. aureus ATCC 29213 (4)		3	1		*P. aeruginosa* ATCC 27853 (5)	5			
E. faecalis ATCC 29212 (4)				4	*E. coli* ATCC 25922 (5)	5			
S. pneumoniae ATCC 49619 (1)			1						

CONCLUSIONS

- Pexiganan demonstrated potent activity against contemporary Gram-negative and -positive pathogens isolated from DFI (in 2013) as well as a select group of resistant pathogens from various infection sites.
- This spectrum of activity included isolates with mechanisms of resistance to many currently available antimicrobials.
- The results of this study demonstrated that the *in vitro* activity of pexiganan against this contemporary collection of isolates was similar to that previously reported by Ge et al in 1999.
- The "all organism" MIC_{90} of 32 µg/mL for the DFI isolates for pexiganan in this study was 250 times below the concentration of pexiganan (free base) in the cream/ delivery vehicle, indicating that the achievable topical levels of pexiganan should be sufficient to inhibit most infecting organisms.
- Further study in infected patients with DFI as well as other wound/skin infections for whom topical therapy is appropriate is warranted.

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