In Vitro Activity of Pexiganan Tested Against Pathogens with Elevated MICs to Topical Antimicrobials

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ABSTRACT

Background: Pexiganan (PEX), a 22-amino acid synthetic analogue of peptide magainin II, is in Phase 3 clinical trials as a topical antibiotic cream for treatment of mild infections of diabetic foot ulcers (DFI). The aim of this study was to evaluate in vitro activity of PEX against bacteria selected as having elevated MICs to topical agents.

Methods: PEX was susceptibility tested against bacterial isolates (110 total from 2004-2013) primarily selected as having elevated MIC values (NWT; compared to wildtype [WT] distributions) to bacitracin (B), polymyxin (PB), neomycin (N), mupirocin (M), retapamulin (R), fusidic acid (FA), or gentamicin. Isolates were mostly from skin and soft tissue infections (SSTIs). MIC testing used CLSI broth microdilution reference methods in cation-adjusted Mueller-Hinton broth.

Results: A narrow range of PEX MIC values (4-32 µg/mL) against *S. aureus* was observed (mode, MIC_{50} and MIC_{90} values were 16 µg/mL). The PEX mode, MIC_{50} and MIC_{90} value for the subsets of isolates with NWT MIC values to B and N (n=14), FA (n=11), M (n=12) and R (n=11) were each at 16 µg/mL. For coagulase-negative staphylococci (CoNS), the PEX mode, MIC_{50} and MIC_{90} were 4, 4, and 8 µg/mL, respectively. The PEX mode and MIC_{50} for each CoNS subset with NWT MIC values was also 4 and 4 µg/mL. For enterococci, the lowest PEX MIC values (8 µg/mL) occurred with *E. faecium; E. faecalis* isolates exhibited MIC values that ranged from 128-256 µg/mL. β-hemolytic streptococci showed PEX range of 4-64 µg/mL with MIC_{50} and MIC_{90} values of 16, and 64 µg/mL. PEX MIC_{50} values ranged from 8-64 µg/mL for NWT subsets of β-hemolytic streptococci. For viridans group streptococci, MIC values for PEX varied by species with highest values occurring for *S. oralis.*

Conclusions: PEX was highly active against a broad-spectrum of bacteria selected to have elevated MIC values to topical antimicrobials including B, PB, N, M, R, FA, or gentamicin, and there was absence of cross-resistance. The PEX MIC_{90} value for the organism groups studied was well below the concentration of PEX free base (8000 µg/mL) in the cream being studied in Phase 3 clinical trials, indicating PEX levels should be sufficient to inhibit potential infecting organisms in superficial SSTIs amenable to a topical antimicrobial. Pexiganan is a potentially important therapeutic agent in the current environment of emerging multi-drug resistant pathogens.

INTRODUCTION

Magainins are broad-spectrum cationic peptides which selectively damage bacterial membranes through mechanisms that make resistance development to these agents by bacteria extremely difficult. Pexiganan is a 22-amino acid synthetic analogue of peptide magainin II undergoing Phase 3 clinical trial 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 (NCT01594762 and NCT01590758).

In this study, the in vitro activity of pexiganan was evaluated against bacterial strains primarily selected as having elevated MICs to currently used or investigational topical antibiotics including bacitracin, polymyxin, neomycin, mupirocin, retapamulin, fusidic acid, and gentamicin.

MATERIALS AND METHODS

Organism collection: A total of 110 bacterial isolates from 2004-2013 were selected as having elevated MICs (compared to wild type distribution) to the topical antibiotics bacitracin, polymyxinB, neomycin, mupirocin, retapamulin, fusidic acid and gentamicin. Strains were primarily from skin and soft tissue infections but some blood isolates were included for the less common isolate types.

Susceptibility testing: Broth microdilution MIC testing was performed according to Clinical and Laboratory Standards Institute (CLSI) standardized methods using MIC panels produced by JMI Laboratories (North Liberty, Iowa, USA). Media utilized were cation-adjusted Mueller-Hinton broth (CA-MHB) supplemented with 2.5-5% lysed horse blood for streptococcal testing. Interpretive criteria for comparator antimicrobials were those as published by CLSI. Quality control (QC) was performed per CLSI M07-A10 and CLSI M100-S25 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 MIC distributions for pexiganan when tested against staphylococci and β-hemolytic streptococci with elevated (non-wild type [NWT]) MIC values against the topical antimicrobial agents; bacitracin, fusidic acid, mupirocin, neomycin and retapamulin are presented in **Table 1**. A narrow range of MIC values for pexiganan (4-32 µg/mL) against all 48 *S. aureus* strains was seen. The mode, MIC_{50} and MIC_{90} value was 16 µg/mL.
- The mode, MIC_{50} and MIC_{90} value for the subsets of isolates with NWT MIC values to bacitracin and neomycin (n=14), fusidic acid (n=11), mupirocin (n=12) and retapamulin (n=11) were also each individually at 16 µg/mL (**Table 1**).
- The mode, MIC_{50} and MIC_{90} values for the resistant subgroups indicates a lack of cross-resistance to other topical antibiotics in *S. aureus* (**Table 1**).
- For the coagulase-negative staphylococci (CoNS), the mode, MIC_{50} and MIC_{90} were 4, 4, and 8 µg/mL, respectively (**Table 1**).
- The mode and MIC_{50} for each of the subsets with NWT MIC values was also 4 and 4 µg/mL (too few isolates to calculate a MIC_{90}). The exception was one isolate with elevated MICs to both bacitracin and neomycin (MIC to pexiganan, 8 µg/mL; **Table 1**).
- The β-hemolytic streptococci exhibited a pexiganan MIC range of 4-64 µg/mL with a mode, MIC_{50} and MIC_{90} values of 64, 16, and 64 µg/mL. The higher pexiganan MIC values (64 µg/mL) were *S. pyogenes. S. agalactiae* MICs were 4-32 µg/mL (**Table 1**).
- Pexiganan MIC_{50} values ranged from 8-64 µg/mL for the various subsets of β-hemolytic streptococci with NWT MIC values to the other agents (**Table 1**).
- The MIC distributions for pexiganan when tested against enterococci and viridans group streptococci with NWT MIC values against bacitracin, fusidic acid, mupirocin, neomycin and retapamulin are presented in **Table 2**.
- For the enterococci, the lowest pexiganan MIC values (8 µg/mL) occurred with the three *E. faecium* isolates. The 9 *E. faecalis* isolates exhibited MIC values ranging from 128-256 µg/mL (**Table 2**).
- For the viridans group streptococci, the MIC values for pexiganan varied by species. *S. constellatus* demonstrated the lowest pexiganan MIC value (2 µg/mL), *S. mitis* MIC values were 64 µg/mL, and *S. oralis* (>1024 µg/mL; **Table 2**).
- The MIC distributions for pexiganan when tested against 15 isolates of Enterobacteriaceae selected as NWT to polymyxin B are presented in **Table 3**.
- The mode and MIC_{50} value for pexiganan was 32 and 64 µg/mL, respectively. The three isolates with the highest pexiganan MIC values (1024 - >1024 µg/mL) exhibited polymyxin B MIC values from 31.3 - >31.3 µg/mL (**Table 3**).
- The MIC distributions for pexiganan when tested against non-fermentative bacilli selected as non-susceptible to polymyxin B are presented in **Table 3**. Pexiganan MIC values ranged from 16-32 µg/mL for *P. aeruginosa* and were at 8 µg/mL for *A. baumannii*.

Table 1. MIC distribution for pexiganan when tested against staphylococci and β-hemolytic streptococci with elevated MIC results against currently available topical antibiotics[a].

Organism group / (No. tested) Antimicrobial agent	No. of strains at MIC (µg/mL): ≤1	2	4	8	16	32	64	128	256	512	1024	>1024	MIC_{50}	MIC_{90}
S. aureus (48)			1	13	33	1							16	16
BAC and NEO (14)				4	10								16	16
FUS (11)			1		10								16	16
MUP (12)				5	6	1							16	16
RETAP (11)				4	7								16	16
CoNS (14)[b]		2	10	1	1								4	8
BAC and NEO (1)				1									8	-
BAC only (2)			2										4	-
FUS (3)			3										4	-
MUP (3)		1	1		1								4	-
RETAP (5)		1	4										4	-
β-hemolytic streptococci (10)[c]			1	3	1	1	4						16	64
BAC and NEO (2)				1			1						8	-
NEO only (2)					1	1							16	-
FUS (3)			1	1			1						8	-
MUP (2)							2						64	-
RETAP (1)				1									8	-

a. BAC = bacitracin elevated MIC >100 µg/mL; FUS = fusidic elevated MIC > 4 µg/mL (staphylococci) or >16 µg/mL (streptococci); MUP = mupirocin elevated MIC >8 µg/mL; NEO = Neomycin elevated MIC >8 µg/mL; RETAP = retapamulin elevated MIC >4 µg/mL.
b. Includes *S. capitis* (1), *S. epidermidis* (10), *S. haemolyticus* (2), *S. hominis* (1).
c. Includes *S. agalactiae* (5), *S. pyogenes* (4), Group G streptococcus (1).

Table 2. MIC distribution for pexiganan when tested against enterococci and viridans Group streptococci with elevated MIC results against currently available topical antibiotics[a].

Organism group / (No. tested) / Antimicrobial agent	No. of strains at MIC (µg/mL): ≤1	2	4	8	16	32	64	128	256	512	1024	>1024	MIC_{50}	MIC_{90}
Enterococcus spp. (12)[b]				3				3	6				128	256
BAC and NEO (3)								2	1				128	-
NEO only (1)				1									8	-
FUS (2)				1					1				8	-
MUP (1)									1				256	-
RETAP (5)				1				1	3				256	-
Viridans Gr. Streptococci (5)[c]		1					2					2	64	-
NEO only (1)												1	>1024	-
FUS (2)							1					1	64	-
MUP (2)		1					1						2	-

a. BAC = bacitracin elevated MIC >100 µg/mL; FUS = fusidic elevated MIC > 4 µg/mL (staphylococci) or >16 µg/mL (streptococci); MUP = mupirocin elevated MIC >8 µg/mL; NEO = Neomycin elevated MIC >8 µg/mL; RETAP = retapamulin elevated MIC >4 µg/mL.
b. Includes *E. faecalis* (9), *E. faecium* (3).
c. Includes *S. constellatus* (1), *S. mitis* group (2), *S. oralis* (2).

Table 3. MIC distribution for pexiganan when tested against Gram-negative bacilli with elevated MIC results against polymyxin B (>4 µg/mL).

Organism group / (No. tested)	No. of strains at MIC (µg/mL): ≤1	2	4	8	16	32	64	128	256	512	1024	>1024	MIC_{50}	MIC_{90}
Enterobacteriaceae (15)				1	1	4	2	2	1	1	2	1	64	1024
E. coli (2)				1								1	-	-
E. aerogenes (2)						1		1					-	-
E. cloacae (3)										1	2		-	-
K. pneumoniae (8)					1	3	2	1	1				-	-
P. aeruginosa (3)					2	1							-	-
A. baumannii (3)				3									-	-
All Gram-negative bacilli (21)				4	3	5	2	2	1	1	2	1	32	1024

CONCLUSIONS

- Pexiganan was highly active against a broad spectrum of bacteria selected to have elevated MIC values to a variety of topical antimicrobials including bacitracin, fusidic acid, mupirocin, neomycin, polymyxin B and retapamulin. Pexiganan also showed activity against gentamicin-susceptible and gentamicin-resistant isolates.
- There was a lack of cross-resistance that occurred between pexiganan and the topical antimicrobials tested. For example, for all *S. aureus*, the pexiganan mode, MIC_{50}, and MIC_{90} values for subsets of isolates with elevated MIC values to the various topical antimicrobials was 16 µg/mL.
- The MIC_{90} value for each of the organism groups studied was below the concentration of pexiganan free base (8000 µg/mL) in the cream that is being studied in Phase 3 clinical trials, indicating the levels of pexiganan should be sufficient to inhibit most infecting organisms in superficial SSTIs amenable to a topical antimicrobial.
- Pexiganan is a potentially important therapeutic agent in the current environment of emerging multi-drug resistant pathogens and further study of pexiganan in infections where resistant bacteria may be encountered is warranted.

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