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In Vitro Spectrum of Pexiganan Activity When Tested against Pathogens from Diabetic Foot Infections and with Selected Resistance Mechanisms

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Pexiganan, a 22-amino-acid synthetic cationic peptide, is currently in phase 3 clinical trials as a topical antimicrobial agent for the treatment of mild infections associated with diabetic foot ulcers. Bacterial isolates from the 2013 SENTRY Antimicrobial Surveillance Program designated as pathogens from diabetic foot infections (DFI) and Gram-negative and -positive pathogens from various infection types that harbored selected resistance mechanisms/phenotypes were tested against pexiganan in reference cation-adjusted Mueller-Hinton broth. The MIC_{50} and MIC_{90} against all organisms tested from DFI were 16 and 32 μg/ml, respectively. *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter koseri*, *Enterobacter cloacae*, *Acinetobacter* species, and *Pseudomonas aeruginosa* MIC values ranged from 8 to 16 μg/ml. Pexiganan MIC values among *Staphylococcus aureus* (methicillin-resistant *S. aureus* [MRSA] and methicillin-susceptible *S. aureus* [MSSA]), beta-hemolytic streptococci, and *Enterococcus faecium* ranged from 8 to 32 μg/ml. Pexiganan activity was not adversely affected for members of the family *Enterobacteriaceae* or *P. aeruginosa* that produced β-lactamases or resistance mechanisms to other commonly used antimicrobial agents. Decreased susceptibility to vancomycin did not affect pexiganan activity against *S. aureus* or *E. faecium*. *Enterococcus faecalis* appears to be intrinsically less susceptible to pexiganan (MIC, 32 to 256 μg/ml). The "all organism" MIC_{90} of 32 μg/ml for pexiganan in this study was >250-fold below the pexiganan concentration in the cream/delivery vehicle being developed for topical use.

Magainans are broad-spectrum antimicrobial agents found in animals that provide innate immunity to defend against microbes in the environment (1–3). These cationic peptides selectively damage bacterial membranes through mechanisms that make the development of resistance to these agents by bacteria extremely difficult (1, 2). Many antimicrobial peptides exist in nature; protegrins and defensins are examples (3–5). Pexiganan is a 22-amino-acid synthetic analogue of peptide magainin II undergoing phase 3 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 (ClinicalTrials.gov registration numbers NCT01594762 and NCT01590758).

Common bacterial pathogens associated with mild (usually treated with oral agents) diabetic foot infections (DFI) include *Staphylococcus aureus* and *Streptococcus* spp. (6). In moderate to severe infections, *S. aureus*, *Streptococcus* spp., members of the family *Enterobacteriaceae*, and obligate anaerobes are the common bacterial pathogens (6). In a prospective randomized clinical trial of severe DFI, enterococci and *Pseudomonas aeruginosa* occurred in approximately 10 and 20% of patients, respectively (7). Pexiganan has been shown to have activity against many of the above-mentioned pathogens (8–10).

The available susceptibility profiles for pexiganan were published in the late 1990s (8–10). To determine whether the current susceptibility profile for pexiganan is unchanged from that previously demonstrated, this study was performed to establish the contemporary activity of pexiganan against DFI isolates and pathogens having newer types of resistance.

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MATERIALS AND METHODS

Organisms. We selected 46 bacterial isolates from the global SENTRY Antimicrobial Surveillance Program (2013) that were designated by the investigational site as pathogens in DFI. These isolates included *Enterobacteriaceae* (15 isolates, including *Escherichia coli* [6 isolates], *Enterobacter cloacae* [2 isolates], *Citrobacter* species [1 isolate], *Proteus vulgaris* [1 isolate], *Morganella morganii* [2 isolates], *Klebsiella pneumoniae* [2 isolates], and *Serratia marcescens* [1 isolate]), *Pseudomonas aeruginosa* (6 isolates), *Acinetobacter baumannii* (1 isolate; resistant to ≥4 antimicrobials), *Streptococcus agalactiae* (2 isolates), *Streptococcus pyogenes* (1 isolate), *Enterococcus faecium* (1 isolate), and *Staphylococcus aureus* (20 isolates; comprised of 12 methicillin-susceptible *S. aureus* [MSSA] isolates and 8 methicillin-resistant *S. aureus* [MRSA] isolates).

We selected an additional collection of 63 Gram-positive and -negative isolates from various other infection types and harboring characterized resistance mechanisms and phenotypes. The isolates harboring resistance genotypes/phenotypes included the following. *S. aureus* (10 isolates; comprised of 2 VRSA [vancomycin-resistant *S. aureus*] isolates, 1 hVISA [heterogeneous vancomycin-intermediate *S. aureus*] isolate, 1 VISA [vancomycin-intermediate *S. aureus*] isolate, 4 community-acquired *S. aureus* [USA300] isolates, and 2 hospital-acquired *S. aureus* [USA100] isolates), vancomycin-resistant phenotypes of enterococci (10 isolates; consisting

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

Organism (no. of isolates tested)	No. of strains with the following MIC (μg/ml) (cumulative %):									MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
	≤4	8	16	32	64	128	256	512	>512		
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)								
E. faecium (1)		1 (100.0)								8	
Enterobacteriaceae (15)		10 (66.7)	1 (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)						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, beta-hemolytic streptococci (includes *S. agalactiae* [2 isolates] and *S. pyogenes* [1 isolate]).
[b] IPP, indole-positive *Proteae* (includes *M. morganii* [2 isolates] and *P. vulgaris* [1 isolate]).
[c] Other includes *Citrobacter koseri* (1 isolate), *E. cloacae* (2 isolates), and *S. marcescens* (1 isolate; MIC, >512 μg/ml).

of 4 *E. faecalis* isolates [2 VanA isolates and 2 VanB isolates] and 6 *E. faecium* isolates [3 VanA isolates and 3 VanB isolates]), and *Enterobacteriaceae*. The 37 *Enterobacteriaceae* isolates consisted of 15 *K. pneumoniae* isolates, 13 *E. coli* isolates, 5 *E. cloacae* isolates, and 4 *K. oxytoca* isolates. The *Enterobacteriaceae* isolates included isolates containing CTX-M-2, -14, and -15; DHA-1 and -2; CMY-2 and -6; FOX-5; SHV-12, -27, and -31; OXA-30; KPC-2 and -3; NDM-1 and TEM-10. *P. aeruginosa* (4 isolates; comprising two carbapenem-resistant phenotypes [mechanism not characterized] and one isolate containing IMP-1 and one isolate containing VIM-2) and *A. baumannii* (two multidrug-resistant isolates, both resistant to ≥4 antimicrobial classes) were also included in this collection.

Susceptibility testing. Pexiganan was supplied in powder form by Dipexium Pharmaceuticals. Broth microdilution MIC testing was performed according to Clinical and Laboratory Standards Institute (CLSI) standardized methods (11) using MIC panels produced by JMI Laboratories (North Liberty, IA, USA). The medium utilized was cation-adjusted Mueller-Hinton broth (CA-MHB) (Ca^{2+} at 20 to 25 mg/liter and Mg^{2+} at 10 to 12.5 mg/liter) supplemented with 2.5 to 5% lysed horse blood for streptococcal testing. Interpretive criteria for comparator antimicrobials were those published by CLSI (12). Quality control (QC) was performed per CLSI M07-A9 and CLSI M100-S24 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 (11, 12). Pexiganan quality control MIC occurrences for *S. aureus* ATCC 29213 were 3 values at 16 μg/ml and 1 value at 32 μg/ml; for *E. faecalis* ATCC 29212, 4 values at 64 μg/ml; for *E. coli* ATCC 25922, 5 values at 8 μg/ml; for *P. aeruginosa* ATCC 27853, 5 values at 8 μg/ml; and for *S. pneumoniae* ATCC 49619, 1 value at 32 μg/ml.

RESULTS

The activities of pexiganan against DFI and select resistant isolates are presented in Tables 1 and 2. The overall pexiganan 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 of >32 μg/ml (actual MIC of >512 μg/ml): three indole-positive *Proteae* isolates (two *M. morganii* isolates and one *P. vulgaris* isolate) and one *S. marcescens* isolate (Table 1). Pexiganan MIC values for the DFI *Acinetobacter* species and *P. aeruginosa* isolates were 8 or 16 μg/ml, which was similar to the MIC value obtained with the *P. aeruginosa* ATCC 27853 QC strain.

Among *S. aureus* isolates from DFI (8 MRSA isolates and 12 MSSA isolates), 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 the three beta-hemolytic streptococci from DFI (two *S. agalactiae* isolates and one *S. pyogenes* isolate), with MIC values at 8 or 16 μg/ml. One *E. faecium* strain was susceptible to pexiganan with a MIC value of 8 μg/ml (Table 1).

Among *E. coli* strains from the select group of resistant phenotype/genotypes, those strains producing extended-spectrum β-lactamases (ESBL) or plasmidic AmpC or NDM-1 enzyme 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 wild-type QC strain (Table 2).

Pexiganan was also active against *Klebsiella* strains with various β-lactamase types, including ESBL, plasmidic AmpC, KPC (*Klebsiella pneumoniae* carbapenemase) types, and NDM-1 (Table 2). Pexiganan MIC values ranged from 4 to 32 μg/ml, except for two strains, one with a pexiganan MIC value of 128 (a KPC-2-producing strain) and one with a MIC of >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 multidrug-resistant (MDR) *Acinetobacter* species 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*, MRSA USA300, 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 at 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. Vancomy-

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

Organism (no. of isolates tested)	No. of strains with the following MIC (μg/ml) (cumulative %):								MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
	≤4	8	16	32	64	128	256	>256		
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 two VRSA isolates, one hVISA isolate, one VISA isolate, four community-acquired *S. aureus* (USA300) isolates, and two hospital-acquired *S. aureus* (USA100) isolates.
[b] Includes two phenotypic VanA isolates and two phenotypic VanB isolates.
[c] Includes three VanA isolates and three VanB isolates.
[d] Included among the 37 isolates are those that harbor CTX-M-2, -14, and -15; DHA-1 and -2; CMY-2 and -6; FOX-5; SHV-12, -27, and -31; OXA-30; KPC-2 and -3; and NDM-1 and TEM-10.
[e] Among the four isolates are two carbapenem-resistant phenotypic isolates, one IMP-1-containing isolate, and one VIM-2-containing isolate.
[f] Two MDR isolates (resistant to ≥4 antimicrobial classes).

cin-resistant *E. faecalis* strains, however, showed higher pexiganan MIC values (64 to >256 μg/ml; Table 2).

DISCUSSION

Previous studies have shown that pexiganan exhibits broad-spectrum activity against Gram-positive and -negative bacteria as well as yeasts (8–10). The MIC_{50} and MIC_{90} values for most Gram-positive species (including anaerobic bacteria) were shown to range from 4 to 64 μg/ml with the exception of *E. faecalis* and *Streptococcus sanguis* (8, 9). Fuchs et al. also showed similar results for *E. faecalis* and viridans group streptococci (MIC_{90}, >256 μg/ml) (10). In the study reported here, most Gram-positive isolates exhibited pexiganan MIC values in the range of 16 to 32 μg/ml. The notable exception was *E. faecalis*, which as in the earlier studies, showed MIC values for pexiganan in the range of 64 to >256 μg/ml. Gram-negative antibacterial activity for pexiganan was demonstrated by Ge et al. (8, 9) and Fuchs et al. (10) to be generally similar to its anti-Gram-positive activity with MIC_{90} values ranging from 8 to 64 μg/ml. In this current study, pexiganan also exhibited MIC values in that range against most Gram-negative bacteria. The exceptions were the indole-positive *Proteae* in the DFI collection (3 isolates with MICs of >512 μg/ml) and two isolates of *K. pneumoniae* (2 isolates; one with a MIC at 128 μg/ml and with a MIC at >256 μg/ml).

The mode of action of cationic peptides occurs through an interaction with lipopolysaccharide (LPS) which leads to uptake of the antimicrobial (1, 2, 13). The first step is an interaction of the cationic polypeptide with divalent cation binding sites on surface LPS. Thus, the presence of high levels of cations (calcium or magnesium) in susceptibility test medium may interfere with this interaction, and MIC values may appear higher than would be observed in a medium with lower cation content. Fuchs et al. showed that for *S. aureus* the geometric mean MIC increased from 17.1 to 19.7 μg/ml when comparing staphylococci grown in Mueller-Hinton broth to cation-adjusted Mueller-Hinton broth (CLSI reference method) and mentioned that larger differences were noted with other organisms (10). In our study we chose to use only the CLSI reference medium (cation-adjusted Mueller-Hinton) for testing. Thus, the MIC values generated in our study were biased to a slightly higher value than the MIC values presented in the Fuchs et al. and Ge at al. studies when they tested Mueller-Hinton broth without cation adjustment (8–10). We chose to use the CLSI reference medium for testing, as that is the medium currently applied for routine susceptibility testing in clinical microbiology laboratories, and therefore, our results can be more readily be compared to clinical results that will be generated in the future.

When tested against the contemporary collection of Gram-positive and -negative pathogens isolated from DFI, pexiganan exhibited broad-spectrum activity which was similar to that shown in previous studies (8–10). Further, pexiganan demonstrated potent activity against Gram-positive and -negative isolates selected to contain contemporary resistance mechanisms that present therapeutic dilemmas, such as vancomycin resistance and carbapenemase production (KPC and NDM). These isolates are resistant to many of the currently available antimicrobials.

The *in vitro* activity of pexiganan against this updated collection of wild-type and resistant isolates was similar to that previously reported in 1998 to 1999, indicating that susceptibility to pexiganan among targeted bacteria has not been altered substantially over the last 15 years (8–10). The "all organism" pexiganan MIC_{90} of 32 μg/ml for the DFI isolates in this study was 250 times lower than the concentration of pexiganan in the cream/delivery vehicle that is under development, indicating that the achievable topical levels of pexiganan should be sufficient to inhibit most infecting organisms. Further study is warranted in DFI patients as well as other wound/skin infection patients for whom topical therapy would be appropriate.

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