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In Vitro Activity of Pexiganan and 8 Comparator Antimicrobials against 234 Isolates Including 93 *Pasteurella* spp. and 50 Anaerobic Bacteria Recovered from Animal Bite Wounds

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Introduction

More than 5 million Americans sustain animal bites each year leading to approximately 10,000 hospitalizations and 1% (300,000) of all emergency department visits annually (1, 2). Others are commonly seen as outpatients in primary care physicians' and specialists' offices. However, prior to seeking medical attention, 84% will attempt self-therapy of which 42% will self-administer topical antimicrobial agents that are ineffective in preventing infection (1, 2).

Magainins are cationic peptides, broad-spectrum antimicrobial agents that naturally occur in animals and act as primary defenses against microbial invaders. Antimicrobial peptides selectively damage bacterial cell membranes through mechanisms that are bactericidal and difficult for bacteria to evade. Pexiganan is a 22-amino acid synthetic analogue of peptide magainin II and is currently in a Phase 3 clinical development as a topical agent (pexiganan cream, 0.8%) for mild infections of diabetic foot ulcers.

We examined the comparative *in vitro* activity of pexiganan against a broad selection of aerobic and anaerobic bacteria recovered from infected animal bite wound specimens obtained from patients in the USA, Canada, Sweden, Hungary, Germany and Holland.

Methods

Agar dilution method for anaerobes. The comparator antimicrobial agents were reconstituted according to the procedures described in the CLSI M11-A8 document (3). Stock solutions were stored at -70°C until the day of the test. They were thawed and serial dilutions were prepared and added to molten Brucella agar deeps for preparation of the plates. Drug-free plates were included as growth controls. Concentrations for penicillin were from 8– 0.03 µg/ml; for piperacillin-tazobactam from 32–0.03 µg/ml and for the remainder 16–0.03 µg/ml.

The organisms were taken from the freezer and subcultured onto blood agar plates at least twice for purity and good growth. On the day of testing, they were suspended in Brucella broth to equal the turbidity of the 0.5 McFarland standard and applied to the plates using a Steers replication device that delivers ~2–5 µl per spot, for a final concentration of approximately 10^5 colony forming units (CFU)/spot. The plates were incubated in the anaerobic chamber at 36°C for 44 hours and examined for growth. The MIC is defined as the lowest concentration of antimicrobial agent that completely in inhibited growth or resulted in a major reduction of growth compared to the drug-free control. Pexiganan was tested by the broth microdilution method (see below).

Broth microdilution method for anaerobes and aerobes. Pexiganan was tested by the broth microdilution method according to standard methods (3, 4) with supplemented Brucella broth for the anaerobic organisms because it binds to the calcium in agar destroying its activity. The broth microdilution method was used with and without 5% lysed horse blood as there was some question regarding decreased activity in the presence of blood. However, many anaerobes require blood for growth in broth media, thus both methods were used. However, for the summaries, only the lysed horse blood supplemented tests are reported. Concentrations for pexiganan were serial two-fold dilutions from 512–0.25 µg/ml.

For aerobic organisms, Mueller Hinton broth supplemented with 5% lysed horse blood was used for testing all of the drugs. Pexiganan was reconstituted in water and serially diluted in Mueller Hinton broth with 5% lysed horse blood. The comparator drugs were reconstituted according to the manufacturers' instructions. The Quick-Spense apparatus was used to dispense 100 µl of the dilutions into 96 well microtiter trays, which were immediately placed into the -70°C freezer for storage. On the day of the test, they were removed from the freezer and thawed at room temperature.

The anaerobic isolates were suspended in Brucella broth to equal the turbidity of the 0.5 McFarland standard, further diluted 1:15 in saline before adding 10 µl to each well for a final concentration of approximately 1 X 10^5 CFU per well. The plates were incubated in the anaerobic chamber at 36°C for 44 hours and examined for growth using an inverted mirror. The MIC was the lowest concentration that completely inhibited growth or caused a marked reduction of growth compared to the drug-free control well.

The aerobic strains were suspended in saline to equal the 0.5 McFarland standard and further diluted 1:30 in saline and added to the trays using a 96-pronged inoculation device that delivered 10 µl to each well for a final concentration of approximately 5 X 10^4 CFU/well. The plates were incubated in an ambient atmosphere at 35°C for 20 hours. The *E. corrodens* test plates were incubated in 5% CO_2 for 48h.

After incubation, the plates were examined using an inverted mirror. The MICs were read and recorded and the $MIC_{50/90s}$ were calculated. The susceptibility of test isolates to the comparator agents was determined per the interpretive breakpoints (4, 5). Interpretive breakpoints for pexiganan have not been defined.

The quality control strains, *Bacteroides fragilis* ATCC 25825 and *Clostridium difficile* ATCC 700057 were included each day of testing anaerobes, and *Staphylococcus aureus* ATCC 29213 and *E. coli* ATCC 25922 were included with the aerobic testing.

References

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Results

Table 1. *In vitro* activities of pexiganan and comparator antimicrobial agents against aerobic organisms(µg/ml)

Organism (no.) / Agent	Range	MIC_{50}	MIC_{90}
Pasteurella multocida ssp. *multocida* (31)			
Pexiganan	4–128	16	32
Penicillin	≤0.03–0.125	0.125	0.125
Amox/clav	0.06–0.25	0.25	0.25
Ceftriaxone	≤0.03–≤0.03	≤0.03	≤0.03
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	4–>16	16	16
Doxycycline	0.125–1	0.25	0.25
Moxifloxacin	≤0.03–≤0.03	≤0.03	≤0.03
P. multocida ssp. *septica* (22)			
Pexiganan	4–32	16	16
Penicillin	≤0.03–0.125	0.125	0.125
Amox/clav	0.06–0.25	0.25	0.25
Ceftriaxone	≤0.03–≤0.03	≤0.03	≤0.03
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	4–>16	16	16
Doxycycline	0.125–0.25	0.25	0.25
Moxifloxacin	≤0.03–0.06	≤0.03	≤0.03
P. canis (15)			
Pexiganan	4–16	8	16
Penicillin	0.06–0.125	0.06	0.125
Amox/clav	0.125–0.25	0.125	0.25
Ceftriaxone	≤0.03–≤0.03	≤0.03	≤0.03
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	4–8	8	8
Doxycycline	0.125–0.25	0.25	0.25
Moxifloxacin	≤0.03–≤0.03	≤0.03	≤0.03
P. dagmatis (15)			
Pexiganan	4–32	8	16
Penicillin	0.06–0.25	0.125	0.2
Amox/clav	0.125–0.25	0.25	0.25
Ceftriaxone	≤0.03–≤0.03	≤0.03	≤0.03
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	4–8	4	8
Doxycycline	0.125–0.25	0.125	0.25
Moxifloxacin	≤0.03–≤0.03	≤0.03	≤0.03
P. stomatis (10)			
Pexiganan	4–8	8	8
Penicillin	0.06–0.25	0.06	0.125
Amox/clav	0.125–0.25	0.125	0.25
Ceftriaxone	≤0.03–≤0.03	≤0.03	≤0.03
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	2–8	4	4
Doxycycline	0.125–0.25	0.25	0.25
Moxifloxacin	≤0.03–≤0.03	≤0.03	≤0.03
Eikenella corrodens (32)			
Pexiganan	4–16	8	8
Penicillin	0.125–4	0.25	0.5
Amox/clav	0.125–2	0.5	2
Ceftriaxone	≤0.03–0.06	≤0.03	≤0.03
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	8–>16	16	>16
Doxycycline	0.25–4	2	4
Moxifloxacin	≤0.03–0.06	≤0.03	≤0.03
Neisseria weaveri (17)			
Pexiganan	1–2	2	2
Penicillin	≤0.03–0.25	0.25	0.25
Amox/clav	≤0.03–0.5	0.25	0.5
Ceftriaxone	≤0.03–0.06	≤0.03	≤0.03
Pip/tazo	≤0.03–0.06	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	2–16	4	8
Doxycycline	≤0.03–0.125	0.125	0.125
Moxifloxacin	≤0.03–≤0.03	≤0.03	≤0.03
N. zoodegmatis (14)			
Pexiganan	1–2	2	2
Penicillin	0.06–0.5	0.125	0.25
Amox/clav	0.06–0.5	0.25	0.5
Ceftriaxone	≤0.03–0.06	≤0.03	≤0.03
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–0.06	≤0.03	≤0.03
Clindamycin	1–4	2	4
Doxycycline	≤0.03–0.25	0.06	0.25
Moxifloxacin	≤0.03–0.06	≤0.03	0.06
Moraxella spp.[a] (16)			
Pexiganan	2–4	2	4
Penicillin	≤0.03–0.5	0.06	0.25
Amox/clav	≤0.03–0.5	0.125	0.5
Ceftriaxone	≤0.03–0.06	≤0.03	≤0.03
Pip/tazo	≤0.03–0.06	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	0.25–4	2	4
Doxycycline	≤0.03–0.5	0.125	0.25
Moxifloxacin	≤0.03–4	≤0.03	0.5
Bergyella zoohelcum (11)			
Pexiganan	2–64	8	16
Penicillin	≤0.03–0.5	≤0.03	0.125
Amox/clav	≤0.03–0.25	≤0.03	0.06
Ceftriaxone	≤0.03–0.125	≤0.03	0.06
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–0.06	≤0.03	≤0.03
Clindamycin	≤0.03–4	0.06	0.125
Doxycycline	0.06–0.25	0.125	0.25
Moxifloxacin	≤0.03–≤0.03	≤0.03	≤0.03

Table 2. *In vitro* activities of pexiganan and comparator antimicrobial agents against anaerobic organisms(µg/ml)

Organism (no.) / Agent	Range	MIC_{50}	MIC_{90}
Bacteroides pyogenes (15)			
Pexiganan	2–128	8	64
Penicillin	≤0.03–8	≤0.03	8
Amox/clav	≤0.03–0.5	≤0.03	0.5
Ceftriaxone	0.06–16	0.125	16
Pip/tazo	≤0.03–0.25	≤0.03	0.125
Meropenem	≤0.03–0.125	≤0.03	0.06
Clindamycin	≤0.03–2	≤0.03	0.125
Doxycycline	≤0.03–4	0.06	2
Moxifloxacin	0.125–0.25	0.125	0.25
Linezolid	2–4	2	2
Metronidazole	0.125–1	0.25	0.5
Prevotella heparinolytica (16)			
Pexiganan	4–128	32	128
Penicillin	≤0.03–2	0.06	0.06
Amox/clav	0.125–1	0.125	0.25
Ceftriaxone	0.125–0.25	0.25	0.25
Pip/tazo	0.06–0.125	0.06	0.125
Meropenem	0.06–0.125	0.06	0.125
Clindamycin	≤0.03–>16	≤0.03	>16
Doxycycline	≤0.03–4	0.06	4
Moxifloxacin	0.25–0.25	0.25	0.25
Linezolid	0.25–2	2	2
Metronidazole	0.06–0.25	0.125	0.25
Fusobacterium canifelinum (10)			
Pexiganan	8–32	16	32
Penicillin	≤0.03–≤0.03	≤0.03	≤0.03
Amox/clav	0.06–0.06	0.06	0.06
Ceftriaxone	0.06–0.5	0.125	0.5
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	≤0.03–0.125	0.06	0.125
Doxycycline	≤0.03–0.25	0.06	0.25
Moxifloxacin	>16–>16	>16	>16
Linezolid	0.5–1	1	1
Metronidazole	0.06–0.5	0.125	0.5
F. russi (10)			
Pexiganan	0.5–4	1	4
Penicillin	≤0.03–0.06	0.06	0.06
Amox/clav	0.125–0.25	0.125	0.125
Ceftriaxone	0.06–0.5	0.125	0.5
Pip/tazo	≤0.03–≤0.03	≤0.03	≤0.03
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03
Clindamycin	≤0.03–0.06	0.06	0.06
Doxycycline	≤0.03–0.25	≤0.03	0.06
Moxifloxacin	8–16	8	8
Linezolid	1–1	1	1
Metronidazole	0.06–0.5	0.125	0.5

[a] *Moraxella* spp.: *Moraxella canis* (11), *M. cunilculi* (1) *and M. lacunata* (4).
Abbreviations: Amox/clav, amoxicillin-clavulanate; Pip/tazo, piperacillin-tazobactam

The MICs of 50% and 90% and the ranges are shown in Tables 1 and 2. MIC_{90} values were not recorded for any species or organism group where fewer than 10 isolates were tested.

Before starting this study, we tested *Bacteroides fragilis* by the agar dilution method and found very high pexiganan MICs; thus we repeated testing using Brucella broth with and without 5% lysed horse blood. The agar inactivated the pexiganan resulting in MICs that were 32– >512 µg/ml; the broth microdilution MICs were in a range similar to what was reported in previous studies using a broth microdilution method.

Because many of the non-*Bacteroides* anaerobic organisms grew poorly or not at all in the blood-free broth microdilution, we reported only the results from the blood supplemented pexiganan tests. In the cases where the organism grew in the blood-free broth, the MICs obtained in the blood supplemented broth tended to be one dilution higher in some cases, although in others, they were the same and rarely lower.

The comparator antimicrobial agents that showed the most resistance included clindamycin, moxifloxacin, doxycycline and in Gram-negative species, the beta-lactam agents. There was no apparent relationship between pexiganan MICs and resistance to any of these agents. This can be seen in the line-listing in the data tables.

Discussion

Epidemic rates of animal bite injuries and infections are not surprising since human-animal contact is a daily occurrence for most people worldwide. Settings for this exposure vary from farms to domestic pets to feral animals with bite injuries caused by a wide variety of domestic and wild animals. The Humane Society of the United States estimates that in 2015 in the United States 65% (79.7 million) households own a pet including 163.6 million dogs and cats (ref: http://www.humanesociety.org/issues/pet_overpopulation/facts/pet_ownership_statistics.html, referenced 6-08-16).

Approximately 4.5 million dog bites occur each year in the United States. Almost 1 out of 5 bites becomes infected (ref: http://www.cdc.gov/features/dog-bite-prevention/, referenced 6-08-16). Among children, the rate of dog-bite–related injuries is highest for those 5 to 9 years old. Children are more likely than adults to receive medical attention for dog bites. Men are more likely than women to be bitten by a dog. Roughly 60% of animal bites are related to dogs, with 10–20% attributed to cats. Cat bites are more common in women and the elderly.

Most of these wounds are minor injuries, go unreported and the patients do not seek medical attention. In industrialized countries, most patients with moderate to severe bite injuries will seek some form of medical attention whether in an Emergency Department (ED) or in a physician's office. This results in more than 5 million bites per year, of which 800,000 seek medical attention including 300,000 ED visits and 100,000 hospitalizations in the US that often requires antimicrobial therapy.

The vast majority of patients initiate some form of self-therapy especially washing the wound with soap and water and additionally often employing non-effective topical agents in an effort to prevent infection and avoid medical visitation. Unfortunately, these agents do not have a spectrum of activity that covers the variety of potential pathogens found in the oral flora of the biting animal (1, 2). Pexiganan is a topical agent in Phase 3 clinical trials for mild infections of diabetic foot ulcers with a concentration of 8,000 µg/ml of the active compound. *Pasteurella* spp. are the most commonly recognized animal bite pathogens and are present in 75% of cat bite wound infections and 50% of dog bite wound infections. Our in vitro studies with pexiganan have evaluated its activity against 93 strains of Pasteurella species and found MIC_{90s} of 8–16 µg/ml with the highest MIC of 128 µg/ml. Given the 8,000 µg/ml cream concentration, it has an excellent multi-fold therapeutic margin. Additionally, we studied a wide variety of other commonly isolated aerobic and anaerobic animal bite wound pathogens and found them to be even more or equally susceptible to pexiganan.

These data suggest a potential therapeutic role of this new topical agent in the initial management of animal bite wounds.

Conclusions

- **Pexiganan was highly active against a broad spectrum of aerobic and anaerobic bacteria recovered primarily from animal bite wounds in humans.**
- **There was no apparent relation to resistance seen with any of the comparator antimicrobials.**
- **The concentration of pexiganan in the cream is 8,000 µg/ml, more than 60X the highest MIC obtained that was required to inhibit the bite wound isolates and is likely sufficient to cover all organisms present in the infected site.**
- **Pexiganan shows great potential as an adjunct for treating animal bite wounds.**