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Pexiganan Acetate

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Contents

Abstract

▲ Pexiganan acetate (MSI 78) is a synthetic cationic peptide (22 amino acids) with antibacterial activity. It is an analogue of magainin 2, which is a host defence peptide isolated from frog skin.

▲ The drug is thought to act by disturbing the permeability of the cell membrane or cell wall.

▲ Pexiganan acetate has good *in vitro* activity against Gram-positive and Gram-negative aerobes; 99% of strains were susceptible to the agent using a break-point of 64 mg/L. 89 to 97% of anaerobes were susceptible to pexiganan acetate using the same break-point.

▲ After 7 passages *in vitro*, there was no evidence of resistance to pexiganan acetate among 2 strains of *Staphylococcus aureus*.

▲ In 2 phase III multicentre randomised double-blind trials in diabetic patients with infected foot ulcers, both topical pexiganan acetate 1% and oral ofloxacin 800 mg/day achieved clinical cure or improvement in about 90% of patients.

▲ Eradication of pathogens in the 2 studies was achieved in 82% of ofloxacin recipients and 66% of pexiganan acetate recipients at the end of therapy.

▲ Limited data indicate that pexiganan acetate is well tolerated.

Features and properties of pexiganan acetate (MSI 78)	
Indication	
Infected foot ulcers in patients with diabetes	
Mechanism of action	
Antibacterial	Disturbs cell membrane permeability
Dosage and administration	
Usual dosage in clinical trials	1% formulation
Route of administration	Topical
Frequency of administration	Not stated
Pharmacokinetic properties	
No details available	
Adverse events	
Most frequent	No details available

Gly-Ile-Gly-Lys-Phe-Leu-Lys-Lys-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Lys-Ile-Leu-Lys-Lys-NH_2

Pexiganan acetate (MSI 78)

Peptides are now a well recognised part of the host defence of mammals, invertebrates and amphibians. Examples of these peptides include the defensins (predominantly found in mammals), cecropins, abaecin, apidaecins and ceratotoxins (insects), polyphemusins and tachyplesins (crabs) and magainins (amphibians).[1] Although these peptides can differ with respect to their amino acid sequence and secondary structure, they are invariably cationic, form amphipathic structures (i.e. secondary structures that have a hydrophobic and a hydrophilic face)[1,2] and cause lysis of the pathogen cell wall or membrane.[1] In addition to the naturally occurring molecules, many analogues have been synthesised to study the structure-activity relationship of these peptides.[1]

Magainin 2 is a 23-amino acid cationic peptide isolated from skin of the African clawed frog (*Xenopus laevis*). It has a broad spectrum of activity against Gram-positive and Gram-negative bacteria, as well as having antitumour activity. Pexiganan acetate (MSI 78) is a synthetic 22-amino acid analogue of magainin 2 which is being investigated for the topical treatment of infected foot ulcers in diabetic patients. The drug was originally tested in the treatment of impetigo; however, further research in this indication was abandoned after phase III data showed clinical improvement in 75% of controls because of proper hygiene methods.[2]

Foot infections are a common problem in patients with diabetes. They can be difficult to manage and are a major contributing factor to amputation; an estimated 44 amputations are performed annually per 10 000 patients with diabetes in Ontario, Canada.[3] *Staphylococcus aureus* and facultative streptococci are the main pathogens in milder infections. More serious infections tend to be polymicrobial; other pathogens involved are enterococci, facultative Gram-negative bacteria, anaerobic Gram-positive cocci and *Bacteroides* spp.[4] Oral antibiotic therapy is recommended for mild infection and oral and/or parenteral therapy with surgical intervention and debridement for moderate to severe infection.[3]

1. Pharmacodynamic Properties

Mechanism of Action

Currently, the only information concerning the mechanism of action of the magainins is general and is not specific for pexiganan acetate.

- The magainins have no ordered structure in aqueous solution (fig. 1). However, they form an amphipathic α-helix on interaction with the negatively charged phospholipids present in the cell membrane or wall. The peptide helix is thought to lie parallel to the membrane surface with hydrophobic amino acids on one side and hydrophilic residues on the other.

- Multiple helices are then thought to organise into a higher-order structure.[7] It is this association of multiple helical peptides that disrupts the cell wall or membrane (possibly by forming holes, channels or imperfections) and, in turn, disturbs membrane permeability and membrane potential.[7] It has been suggested that the helices may thin the membrane sufficiently to cause destabilisation[5] or, alternatively, 4 to 6 helices may interact to form temporary membrane-spanning pores (fig. 1).[6]

- Consistent with this mechanism of action, magainin 2 does not seem to interact with a chiral centre such as a receptor or an enzyme. This is supported by the observation that the *d*-enantiomer of magainin 2 retains its antibacterial activity and has similar physicochemical properties to the naturally occurring all-*l* compound.[8,9]

- Structure-activity relationship studies have shown that the antimicrobial activity of the magainin peptides can be boosted; however, this is at the expense of specificity and tends to be accompanied by an increase in haemolytic activity.[1] In the case of

pexiganan acetate, the concentrations required to lyse 5 and 50% of human erythrocytes are 19 and 118 mg/L, respectively.[10]

In Vitro Activity

The *in vitro* activity of pexiganan acetate is presented in figure 2. In common with other cationic peptides, pexiganan acetate does not exhibit the very low minimum inhibitory concentrations (MIC) that characterise modern antibiotics.[1,2] MIC values for cationic peptides tend to be in the range of 1 to 8 mg/L.[2] It has been suggested that this is because these peptides have evolved to act locally at high concentrations.[1] Standard susceptibility break-points for pexiganan acetate have not yet been established, although a susceptibility break-point of ≤64 mg/L has been used in some *in vitro* studies.[11,12]

Gram-Positive Bacteria

- Pexiganan acetate demonstrated good activity against Gram-positive bacteria (fig. 2). MIC values were ≤16 mg/L for all staphylococci tested, with the exception of methicillin-resistant *S. aureus* (MIC range 16 to 64 mg/L). For streptococci, MIC values were generally in the range of 8 to 64 mg/L, except for *S. pyogenes* (MIC range 16 to 256 mg/L) and viridans group streptococci (4 to >256 mg/L).[11] Pexiganan acetate was inactive against *Enterococcus faecalis*.[11,12] Using a break-point of 64 mg/L, 99% of Gram-positive aerobes (n = 393) were susceptible to pexiganan acetate versus 78% to ofloxacin (fig. 3).[12]

Gram-Negative Bacteria

- Pexiganan acetate was also active against a range of Gram-negative bacteria (fig. 2). MIC values tended to lie within a 4 to 32 mg/L range, except for *Enterobacter cloacae* (range 1 to 128 mg/L), *Citrobacter freundii* (8 to >256 mg/L) and *Alcaligenes faecalis* (4 to 64 mg/L).[11] Of a population of 431 Gram-negative aerobes, 99% were susceptible to pexiganan acetate (break-point of 64 mg/L) and 90% to ofloxacin (fig. 3).[12]

- *Burkholderia cepacia* and *Serratia marcescens* are reported to be naturally resistant to cationic peptides;[2] no published data are available concerning the activity of pexiganan acetate against these pathogens.

Anaerobes and Candida albicans

- Pexiganan acetate had strong activity against *Bacteroides* spp. and *Clostridium difficile*, but more variable activity against other anaerobes (fig. 2).[11] Of Gram-positive or Gram-negative anaerobes (n = 61 or 70), 89 to 97% were susceptible to pexiganan

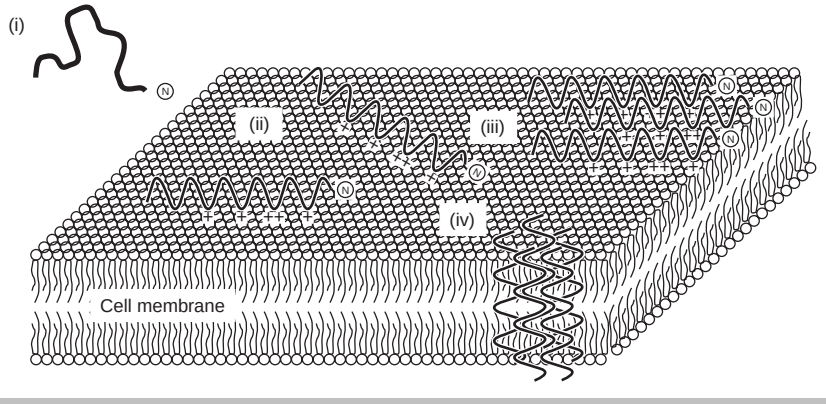

Fig. 1. Schematic representation of the mechanism of action of the magainins. (i) Magainins have no ordered structure in solution, (ii) but form an amphipathic α-helix on interacting with the negatively charged phospholipids of the cell membrane. (iii) Helices are thought to either thin the membrane sufficiently to cause destabilisation[5] or (iv) 4 to 6 α-helices may aggregate and span the membrane temporarily to form a pore, disrupting membrane permeability and leading to cell death.[6,7]

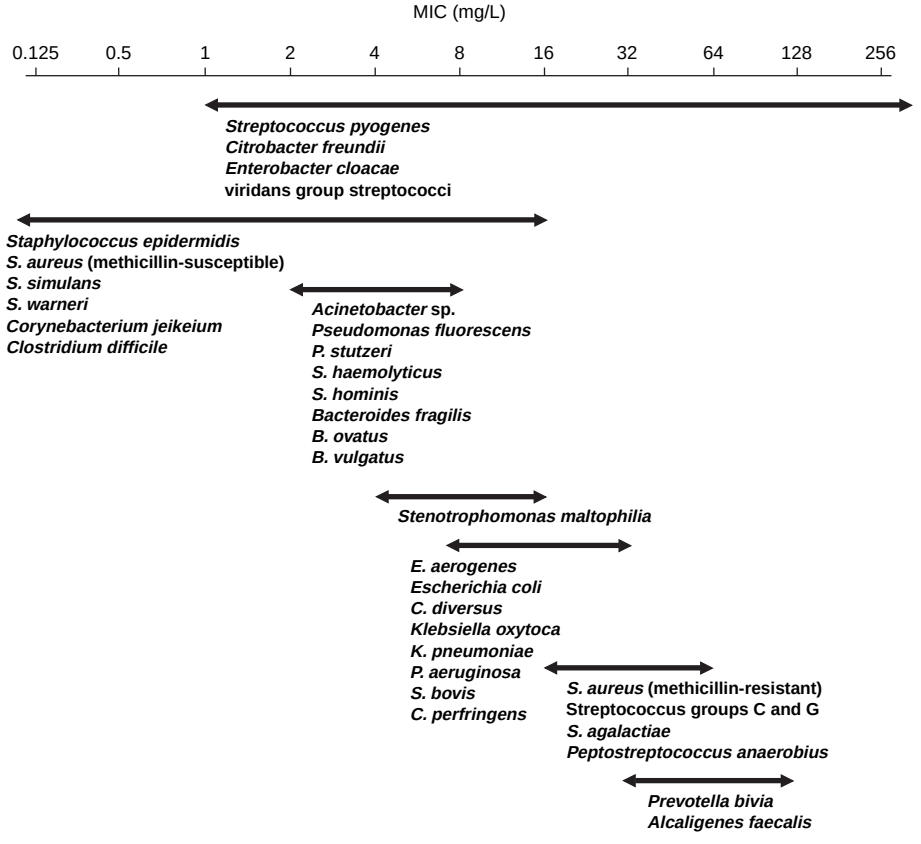

Fig. 2. *In vitro* activity of pexiganan acetate against aerobic and anaerobic bacteria (n = 421). Each horizontal arrow indicates the range of minimum inhibitory concentrations (MIC) for the listed bacteria; the range of MIC values for given bacteria may, however, be smaller than that indicated by the arrow. Only species for which ≥5 isolates were available are depicted. All bacteria were tested by broth microdilution methods in accordance with procedures recommended by the US National Committee for Clinical Laboratory Standards.[11]

acetate (break-point of 64 mg/L) compared with 56 to 76% to ofloxacin.[11,12]

- Pexiganan acetate also has some activity against *Candida albicans*.[11,12] Three of 10 strains of *C. albicans* were inhibited by pexiganan acetate (MIC 64 mg/L) at 24 hours and 2 remained inhibited after 48 hours.[11]

Other In Vitro *Data*

- Pexiganan acetate demonstrated bactericidal activity at ≤2-fold MIC values against 93% of isolates versus 90% for ofloxacin (n = 161).[11]

- There was no evidence of emergence of resistance in 2 strains of *S. aureus* exposed to 7 passages of pexiganan acetate (at 0.5 MIC); MIC values remained constant at 4 to 8 mg/L. One strain was mupirocin sensitive and the other intermediately mupirocin resistant. By comparison, there was a 4- to 128-fold increase in mupirocin MIC values for the same strains after exposure to this agent (at 0.5 MIC).[13]

In Vivo Activity

In Animals

- In a domestic swine model of wound infection, topical pexiganan acetate 2% applied every 8 hours significantly reduced the number of viable gentamicin-sensitive or -resistant *Pseudomonas aerugi-*

nosa by 3 to 5 log units ($p < 0.05$ *vs* vehicle). By comparison, gentamicin significantly reduced microbial counts of gentamicin-sensitive strains (by 4 to 5 log units; $p < 0.05$) but was ineffective against gentamicin-resistant strains.[14]

- In *S. aureus* wound infection in the domestic swine, pexiganan acetate 2% 3 times daily significantly reduced the number of viable organisms by 4 and 2 log units at 24 and 48 hours, respectively, compared with vehicle in one study ($p < 0.05$)[14] and by 1.4 colony forming units/ml at 96 hours after twice daily administration in another study.[15] In the same model, mupirocin 2% reduced microbial counts by 1.5 and 2.5 log units at 24 and 48 hours ($p < 0.05$ *vs* vehicle), respectively,[14] and 5.6 colony forming units/ml at 96 hours.[15]

In Humans

- A single topical dose of pexiganan acetate 0.5, 1 or 2% achieved a significant 3.5 to 4 log reduction in bacterial numbers 1 and 6 hours after application in healthy volunteers ($p < 0.05$ *vs* vehicle). After 24 hours, the 2% concentration was significantly more effective than the 0.5% concentration ($p < 0.01$). In this study, skin flora was transferred from the perineum to the forearm of 45 volunteers and the inoculation sites were occluded using an impermeable plastic film.[16]

2. Pharmacokinetic Profile

Currently there are no published data concerning the pharmacokinetics of pexiganan acetate.

3. Therapeutic Trials

Two phase III multicentre randomised double-blind trials involving a total of 835 diabetic patients with infected foot ulcers have been performed. In both studies, topical pexiganan acetate 1% cream (frequency of administration not stated) was compared with oral ofloxacin 400mg administered twice daily for 14 to 28 days.[17,18] In all patients, foot ulcers were suitable for outpatient management. Currently, both trials are reported as abstracts[17,18] and one abstract presents a combined analysis of both trial results.[17]

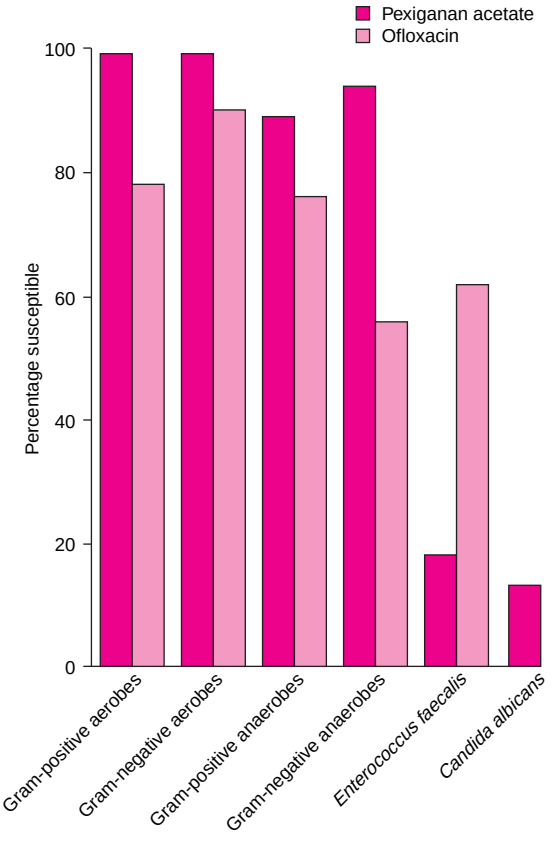

Fig. 3. Percentage susceptibility of 910 clinical isolates to pexiganan acetate and ofloxacin. The susceptibility break-point used for pexiganan acetate was 64 mg/L and the susceptibility breakpoint for ofloxacin was not stated. Ofloxacin was inactive against *C. albicans*.[12]

- Overall, eradication of pathogens was achieved in 82% of ofloxacin recipients and 66% of pexiganan acetate recipients at the end of therapy (no statistical analysis provided).[17] Eradication rates for *S. aureus* were 75% for ofloxacin and 43% for pexiganan acetate;[19] no bacteriological data for other pathogens were provided. Staphylococci, enterococci and streptococci were the most common isolates, with a mean of 2.2 pathogens per positive culture.[17]

- Clinical cure or improvement was achieved in about 90% of patients treated with either pexiganan

acetate or ofloxacin, with no significant differences between treatment groups.[17] Closure occurred in 10 to 15% of wounds after 4 weeks and 18 to 30% of wounds after 6 weeks,[19] again with no statistical differences between treatment groups.[17] In one trial, the incidence of amputation or osteomyelitis at the affected site was reportedly similar in the 2 treatment groups.[18]

4. Tolerability

- In one phase III trial (n = 490),[18] both pexiganan acetate and ofloxacin were reported to be well tolerated, although insomnia occurred significantly more frequently with ofloxacin. No other tolerability data were provided.
- No adverse events were reported in 45 volunteers after a single application of pexiganan acetate 0.5, 1 or 2%.[16]
- *In vitro* data for pexiganan acetate suggest that the drug does have haemolytic activity at concentrations relevant for antibacterial activity (see section 1); however, the implications of this for topical use are unknown.
- No long term tolerability data are available for pexiganan acetate.

5. Pexiganan Acetate: Current Status

Pexiganan acetate is a topical cationic peptide with antibacterial activity. It has shown similar clinical efficacy to that of oral ofloxacin in the treatment of infected foot ulcers in patients with diabetes.

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