Journal of Medical Sciences (2011); 4(2): 62-72

Review Article

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Clinical Applications of Amphibian Antimicrobial Peptides

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Abstract

Frog skin constitutes a rich source of peptides with broad spectrum antimicrobial activity against strains of antibiotic-resistant bacteria and fungi and several hundred such peptides from diverse species have been described. However, their therapeutic potential remains to be realized and no anti-infective peptide based upon their structures has yet been adopted in clinical practice. This review assesses potential clinical applications of nine antimicrobial peptides isolated from frog skin (alyteserin-1c, ascaphin-8, brevinin-1BYa, brevinin-2PRa, brevinin-2-related peptide, brevinin-2-related peptide-ERa, kassinatuerin-1, pseudin-2, and temporin-DRa). The multidrug-resistant microorganisms targeted include the Gram-negative bacteria Acinetobacter baumannii, Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa, the Gram-positive bacterium Staphylococcus aureus, and the opportunistic yeast pathogens Candida spp. Although the naturally occurring peptides show varying degrees of cytotoxicity towards mammalian cells such as erythrocytes, analogs have been developed that retain high antimicrobial potency but are non-hemolytic. Treatment and prevention of acne and periodontal disease are identified as areas in which frog skin antimicrobial peptides might find future applications.

Keywords: Frog skin, antimicrobial peptide, antibiotic-resistant bacteria.

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Introduction

The emergence in all regions of the world of strains of pathogenic bacteria and fungi with resistance to commonly used antibiotics constitutes a serious threat to public health and has necessitated a search for novel types of antimicrobial agent to which the microorganisms have not been exposed[1]. Although effective new types of antibiotics against multidrug-resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) have been introduced or are in clinical trials, the situation regarding new treatment options for infections produced by multidrug-resistant Gram-negative pathogens such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia* is less encouraging[2]. Peptides with potent antibacterial and antifungal activity play an important role in the system of innate immunity that predates adaptive immunity and constitutes the first-line defense against invading pathogens for a wide range of vertebrate and invertebrate species[3]. Anti-infective compounds based upon such peptides are being increasingly considered as potential therapeutic agents[4]. Although development of resistance to antimicrobial peptides has been demonstrated experimentally

in vitro[5], it occurs at rates that are orders of magnitude lower than those observed for conventional antibiotics. Major obstacles to the development of peptide-based anti-infective drugs, particularly if they are to be administered systemically, are their toxicities and their short half-lives in the circulation[6]. However, peptides applied to infected skin or skin lesions in the form of sprays or ointments can penetrate into the *stratum corneum* to kill microorganisms so that future therapeutic applications are more likely to involve topical rather than systemic administration.

Skin secretions from many species of Anura (frogs and toads) contain a wide range of compounds with biological activity, often in very high concentration, that have excited interest because of their potential for drug development [7]. Among these substances are host-defense peptides with broad-spectrum antibacterial and antifungal activities and the ability to permeabilize mammalian cells[8]. Over 20 years have passed since the discovery of the magainins in the skin of African clawed frog, *Xenopuslaevis*. These peptides, identified independently by Michael Zasloff at the National Institutes of Health, Bethesda, U.S.A.[9] and by the group of Dudley H. Williams at the University of Cambridge, U.K.[10], were the first amphibian peptides with antimicrobial activity to be fully characterized. Since that time several hundred such peptides have been isolated from the skin secretions of many other frogs belonging to different families. However, despite showing potent activity against strains of antibiotic-resistant bacteria and against certain pathogenic fungi and protozoa, the potential of these peptides as therapeutic agents has not been realized. No anti-infective peptide based upon their structures has yet been adopted in clinical practice. This review will examine possible clinical application of several well characterized antimicrobial peptides that have been isolated from frog skin. Those peptides with therapeutic potential that have been discovered at the United Arab Emirates University are shown in *Table 1*.

Molecular Properties of Frog Skin Antimicrobial Peptides

Frog skin antimicrobial peptides vary in size from as small as 8up to 48 amino acid residues[11] and a comparison of their amino acid sequences reveals the lack of any conserved domains that are associated with biological activity. However, with few exceptions, these peptides are cationic, generally with a molecular charge between +2 and +6 at pH 7 due to the presence of multiple lysine residues, and contain at least 50% hydrophobic amino acids of which leucine and isoleucine are usually the most abundant. Circular dichroism and NMR studies have shown that they generally lack stable secondary structure in aqueous solutions but have the propensity to form an amphipathic α-helix in the environment of a phospholipid vesicle or in a membrane-mimetic solvent such as 50% trifluoroethanol-water[12]. There is no single mechanism by which peptides produce cell death but their action generally does not involve binding to a specific receptor rather a non-specific interaction with the bacterial cell membrane that results in permeabilization and ultimate disintegration[13]. Consequently, they are usually active against microorganisms resistant to currently licensed antibiotics due to their markedly different mode of action.

The frog skin antimicrobial peptides may be grouped together in peptide families on the basis of limited similarities in amino acid sequence. Skin secretions from a single species frequently contain several members of a particular family that are presumed to have arisen from multiple duplications of an ancestral gene. The molecular heterogeneity of the peptides within a particular family is considerable with a peptide from one species rarely being found with an identical amino acid sequence in another, even when those species are quite closely related phylogenetically[14]. The variation in primary structure is reflected in a wide variability in antimicrobial potencies and specificities for different microorganisms and it has been suggested that this multi-

Table 1. Naturally Occurring Antimicrobial Peptides from Frog Skin with Potential for Development into Potent, Non-Toxic, Anti-Infective Agents for Use Against Antibiotic-Resistant Bacteria

Frog species	Naturally occurring antimicrobial peptide	Primary structure	Microorganism targeted
Midwife toad *Alytesobstetricans*	Alyteserin-1c	GLKDIFKAGLGSLVKGIAAHVAN[a]	Multidrug-resistant *Acinetobacter baumannii* (MDRAB)
Mink frog *Lithobatesseptentrionalis*	Brevinin-2 related peptide	GIWDTIKSMGKVFAGKILQNL[a]	Multidrug-resistant *Acinetobacter baumannii* (MDRAB)
Tailed frog *Ascaphustruei*	Ascaphin-8	GFKDLLKGAAKALVKTVLF[a]	Extended-spectrum β-lactamase (ESBL) *Klebsiella pneumoniae*
Paradoxical frog *Pseudisparadoxa*	Pseudin-2	GLNALKKVFQGIHEAIKLINNHVQ	Antibiotic-resistant *Escherichia coli*
African running frog *Kassinasenegalensis*	Kassinatuerin-1	GFMKYIGPLIPHAVKAISDL I[a]	Antibiotic-resistant *Escherichia coli*
California red-legged frog *Ranadraytonii*	Temporin-DRa	HFLGTLVNLAKKIL[a]	Methicillin-resistant *Staphylococcus aureus* (MRSA)
Green paddy frog *Hylaranaerythraea*	Brevinin-2-related peptide-ERa	GVIKSVLKGVAKTVALGML[a]	Methicillin-resistant *Staphylococcus aureus* (MRSA)
Hokkaido frog *Ranapirica*	Brevinin-2PRa	GLMSLFKGVLKTAGKHIFKNVGGSLLDQAKCKITGEC	Antibiotic-resistant *Pseudomonas aeruginosa*
Foothill yellow-legged frog *Ranaboylii*	Brevinin-1BYa	FLPILASLAAKFGPKLFCLVTKKC	Fluconazole-resistant *Candida* spp.

C-terminal alpha-amidation is denoted by [a].

plicity may provide a broader spectrum of defense against the range of pathogenic microorganisms encountered in the environment[15].

Peptides Active Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin resistance first appeared among nosocomial isolates of *S. aureus* in 1961 and since that time MRSA has emerged to become a major phenotype in hospitals worldwide with a high rate of mortality [16]. MRSA produces an alternative transpeptidase with low affinity for β-lactam antibiotics which results in not only methicillin resistance but *in vivo* non-susceptibility to almost all β-lactam antibiotics. More recently, new strains of MRSA have emerged in the community causing infections in young, otherwise healthy people [17]. In addition to β-lactam resistance, MRSA strains may exhibit multidrug resistance, including non-susceptibility to several other classes of antibiotics such as quinolones, macrolides and sulphonamides[18].

Temporin-DRa, isolated from skin secretions of the California red-legged frog *Ranadraytonii*[19], shows high growth-inhibitory potency against clinical isolates of MRSA (Minimum Inhibitory Concentration, MIC = 8 μM) and has the advantages of ease of synthesis and high solubility[20]. Its therapeutic potency is limited by moderately high hemolytic activity (LC_{50} = 65 μM). However, the analog containing the amino acid substitution Val[7]→ Lys retains activity against MRSA (MIC in the range 8 – 16 μM) but has very low hemolytic activity (LC_{50} > 300 μM)[20]. As well as increasing cationicity, the substitution Val[7]→ Lys decreases amphipathicity by increasing the polar angle θ (the angle

subtended by the positively charged residues) from 100° to 140° thereby delocalizing the positive charge over a greater surface area of the molecule[21].

Brevinin-2-related peptide (B2RP-ERa) was first isolated from skin secretions of the South-East Asian Green Paddy frog *Hylaranaerythraea* (formerly *Ranaerythraea*)[22]. The C-terminally α-amidated peptide shows limited structural similarity to brevinin-2 peptides isolated from other Asian species but lacks the C-terminal cyclic heptapeptide domain (Cys-Lys-Xaa_4-Cys). B2RP-ERa was active against clinical strains of MRSA belonging to different epidemic clonal lineages with MIC values in the range 25 to 50 μM. In time-kill kinetic assays, B2RP at a concentration of 2 x MIC was bacteriostatic but at a concentration of 4×MIC the peptide was bactericidal with 99.9% of bacteria killed within 24 hours. The hemolytic activity of the peptide was relatively low (LC_{50} = 280 μM) (unpublished data).

Peptides Active against Extended-Spectrum β-Lactamase (ESBL) Producing Bacteria

Bacteria which possess extended-spectrum β-lactamases (ESBLs) have the capacity to hydrolyse a broad spectrum of beta-lactam antibiotics, including third generation cephalosporins[23]. Originally observed in *Escherichia coli* and *Klebsiella spp*, ESBL production has now been documented in other Gram-negative bacilli including *Proteus mirabilis*, *Citrobacter-freundii, Shigellasonnei, Serratiamarcescens, Acinetobacter* spp. and *Salmonella* spp. [24]. The epidemiology of ESBL producing *Enterobacter iaceae* is changing with the incidence of community-acquired infections progressively increasing [25]. Treatment of patients with bacterial infections caused by such multi-resistant bacteria is challenging as antibiotic options are becoming increasingly limited.

Ascaphin-8 is a cationic α-helical peptide isolated from skin secretions of the tailed frog *Ascaphustruei* that shows broad-spectrum antibacterial activity but is also moderately toxic to human erythrocytes (LC_{50}=55μM)[26]. All ESBL-producing clinical isolates of *Escherichia coli* (MIC=1.5-6 μM) and *Klebsiella pneumoniae* (MIC=12.5–25μM) strains tested were susceptible to ascaphin-8, as well as a group of miscellaneous ESBL-producing strains (*Citrobacter*, *Salmonella*, *Serratia*, *Shigella* spp.) (MIC≤ 25μM)[27]. Analogs of ascaphin-8 in in which the amino acids at positions 10, 14, or 18 were replaced by lysine retained potent anti-bacterial activity while showing very low hemolytic activity (LC_{50} >500 μM). Unexpectedly, ESBL-producing strains of *Proteus mirabilis* were susceptible toascaphin-8 (MIC=12.5 - 25 μM) although non-ESBL isolates of this organism were resistant to these peptides (MIC>100 μM).

Pseudin-2, a 24 amino-acid-residue antimicrobial peptide first isolated from the skin of the South American paradoxical frog *Pseudisparadoxa*[28], also shows potential for treatment of infections caused by ESBL-producing Gram-negative bacteria, particularly *E. coli*. The naturally occurring peptide has weak hemolytic activity but also relatively low potency against microorganisms. However, analogs of the peptide with increased cationicity and decreased α-helicity showed improved therapeutic properties[29]. [D-Lys^3, D-Lys^{10}, D-Lys^{14}] pseudin-2 showed potent activity against Gram-negative bacteria (MIC against several antibiotic-resistant strains of *E. coli* = 5 μM) but very low hemolytic activity (HC_{50} > 500 μM) and cytolytic activity against L929 fibroblasts (LC_{50} = 215μM). Time-kill studies demonstrated that the analog at a concentration of 1 x MIC was bactericidal against *E. coli* (99.9% cell death after 96 min) but was bacteriostatic against *S. aureus.*

Kassinatuerin-1, a 21-amino-acid C-terminally α-amidated peptide isolated from the skin of the African frog *Kassinasenegalensis*, shows broad-spectrum antimicrobial activity but its therapeutic potential is limited by its relatively high cytolytic activity against mammalian cells[30]. Analogs containing L-lysine substitutions at Gly^7, Ser^{18}, and Asp^{19} displayed increased antimicrobial potency but also increased hemolytic activities. In contrast, the analog with D-lysine at positions 7, 18 and 19 was active

against a range of strongly antibiotic-resistant strains of *E. coli* (MIC = 6 - 12.5 μM) but showed no detectable hemolytic activity at 400 μM. However, the reduction in α-helicity produced by the D-amino acid substitutions resulted in analogs with reduced potencies against Gram-positive bacteria and against the opportunistic yeast pathogen *Candidaalbicans*[31].

Peptides Active Against Multidrug-Resistant *Acinetobacter baumannii* (MDRAB)

There has been a dramatic increase in the number of hospital acquired infections caused by the opportunistic Gram-negative pathogen *A. baumannii* during the past decade [32]. These are typically encountered in immunocompromised and critically ill patients in intensivecare and burns units. However, reports of increasing incidence of community-acquired infections[33] and infections of military personnel with war wounds[34] mean that *A. baumannii* represents a serious threat to public health. Among strains causing nosocomial outbreaks, resistance to fluoroquinolones, aminoglycosides, sulphonamides, third-generation cephalosporins and even carbapenems are common. Treatments with alternative drugs such as polymyxins, particularly colistin (polymyxin E), and the glycylcycline, tigecycline are far from optimal due to concerns with nephrotoxicity regarding colistin and the bacteriostatic nature of tigecycline[35]. Furthermore, increasing use of these antibiotics is already leading to the emergence of resistant strains. The antibiotic resistance of *A. baumannii* arises from a combination of different possible mechanisms: production of hydrolysing enzymes, activation of multi-drug efflux pumps, modification of the drug target, and poor penetration due to loss of porins[36]. These mechanisms are unlikely to reduce the efficacy of antimicrobial peptides.

Alyteserin-1c, isolated from skin secretions of the midwife toad *Alytesobstetricans*[37] displays potent activity against clinical isolates of MDRAB (MIC = 5 - 10 μM; Minimum Bactericidal Concentration, MBC = 5 - 10 μM) while displaying low hemolytic activity against human erythrocytes (LD_{50} = 220 μM)[38]. Increasing the cationicity of alyteserin-1c by the substitution $Glu^4 \rightarrow Lys$ enhanced the potency against MDRAB (MIC= 1.25 - 5 μM; MBC = 1.25 - 5 μM) as well as decreasing hemolytic activity (HC_{50}>400 μM). The bactericidal action of the analog was rapid with more than 99.9% of the bacteria being killed within 30 min at a concentration of 1 x MBC. Increasing the cationicity of [Lys^4]alyteserin-1c further by the additional substitutions of Ala^8,Val^{14} or Ala^{18} by L-Lys did not enhance antimicrobial potency. In an attempt to prepare a long-acting analog of alyteserin-1c suitable for systemic use, a derivative of [Lys^4]alyteserin-1c containing a palmitate group coupled either to the α-amino group at the N-terminus was synthesized. The peptide retained antimicrobial activity against MDRAB but showed dramatically increased hemolytic activity (> 40-fold).

Brevinin-2 related peptide (B2RP), isolated from skin secretions of the mink frog *Lithobatesseptentrionalis*[39], represents a second peptide with therapeutic potential for treatment of MDRAB infections. B2RP potently inhibited the growth of nosocomial isolates of multidrug-resistant *A. baumannii* (MIC = 3 - 6 μM). B2RP also shows relatively high potency (MIC ≤ 25 μM) against Gram-positive and Gram-negative bacteria and against the opportunistic yeast pathogen *C. albicans* but its therapeutic potential is limited by moderate hemolytic activity against human erythrocytes (LC_{50} = 90 μM)[40]. Increasing cationicity of B2RP without changing amphipathicity by the substitution $Asp^4 \rightarrow Lys$ resulted in increased potency against MDRAB isolates (MIC = 1.5 - 3 μM) and a 4-fold increase in potency against *E. coli* (MIC = 6 μM) and 2-fold increases in potency against *S. aureus* (MIC = 12.5 μM) and *Candida albicans* (MIC = 6 μM) without changing significantly hemolytic activity against human erythrocytes (LC_{50} = 95 μM). The analogs [Lys^4, Lys^{18}] B2RP and [Lys^4, Ala^{16} Lys^{18}] B2RP showed reduced potency against *S. aureus* but they retained activity against *A. baumannii* (MIC = 3 - 6 μM) and had very low hemolytic activity (LC_{50} > 400 μM).

There is a growing body of evidence demonstrating that histones, and peptide fragments derived from histones, may play a role in the defence against microorganisms in addition to their "classical" role in chromatin formation[41]. Buforin 2, comprising a fragment of histone H2A, was isolated from an extract of stomach tissue of the toad *Bufobufogargarizans* and its formation may involve the action of pepsin upon the cytoplasmic unacetylated histone H2A that is released into the gastric lumen [42]. The peptide does not produce cell lysis but penetrates the membrane and inhibits cellular functions by binding strongly to DNA and RNA[43]. Buforin 2 was particularly effective (MIC range 0.25 –16 μg/ml) against multidrug-resistant strains of *A. baumanni*[44] and *S. maltophilia*[45] isolated from immunocompromised hospital patients.

Peptides Active Against Azole-Resistant *Candida* spp.

The widespread use of azoles has led to the rapid development of multidrug resistance in *C. albicans* and other *Candida* species, which poses a major problem for antifungal therapy[46]. Patients in ICU, undergoing abdominal surgery[47],orprolonged immunosuppressive therapy for transplants or treatment of malignancy[48], and patients with indwelling devices [49] are particularly at risk for nosocomial *Candida* infections.

Brevinin-1BYa is a cationic α-helical peptide containing an intramolecular disulphide bridge that was first isolated from skin secretions of the foothill yellow-legged frog *Ranaboylii*[50]. As well as showing growth inhibitory activity against a range of reference strains of Gram-positive and Gram-negative bacteria and against clinical isolates of MRSA (MIC = 2.5 μM), the peptide was active against reference strains and clinical isolates of the opportunistic yeast pathogens *C. albicans*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* (MIC≤ 10μM)[51]. However, the therapeutic potential of the peptide, especially for systemic applications, is restricted by its high hemolytic activity against human erythrocytes (LD_{50} = 10 μM). Replacement of the cysteine residues in brevinin-1BYa by serine produced an acyclic analogue with eight-fold reduced hemolytic activity that retained high potency against strains of MRSA (MIC = 5 μM) but activities against yeast species were reduced (MIC in the range 10 – 40 μM). More recently, a cyclic analog of brevinin-1BYa was prepared in which the intramolecular disulphide bridge in the peptide was replaced by a metabolically stable, non-reducible dicarba bond. The resulting compound showed increased antifungal activity (MIC against *C. albicans* = 3 μM) but this advantage was offset by increased hemolytic activity (LD_{50} = 4μM)[52].

Peptides Active Against Antibiotic-Resistant *Pseudomonas aeruginosa*

The opportunistic Gram-negative bacillus *Pseudomonas aeruginosa* is characterized by its intrinsic resistance to several antibiotics and for its abilities to colonize diverse habitats and cause serious disease in vulnerable populations[53]. The bacterium is found in low concentrations amongst the intestinal and skin flora of healthy humans but in compromised hosts, such as immunosuppressed patients and those with neutropenia, burns, cancer, diabetes mellitus and chronic lung disease, it is responsible for life-threatening infections[54]. In particular, *P. aeruginosa* is the major pathogen in the lungs of patients with cystic fibrosis where its survival is enhanced by conversion to biofilm-growing mucoid (alginate-producing) strains[55]. Hospitals represent a reservoir of drug-resistant strains so that nosocomial infections of the respiratory and urinary tracts constitute a growing problem[56].

Brevinin-2PRa, isolated from an extract of the skin of the Hokkaido frog, *Ranapirica,* displayed high potency (MIC values between 6 and 12 μM) against a range of clinical isolates of *P. aeruginosa* with varying degrees of antibiotic resistance and activity was unaffected by NaCl concentrations up to 200mM[57]. The peptide was also active against reference strains of other Gram-negative (*E. coli*, *Enterobacter cloacae*, and *K. pneumoniae*)

and Gram-positive (*S. aureus, S. epidermidis*) bacteria but displayed moderate hemolytic activity (LC = 55 μM).

Peptides of the esculentin-1 family, first identified in the hybrid frog *Ranaesculenta*, comprise 46 amino acid residues but the amidated N-terminal fragment, esculentin-1-(1-18)-peptide (GIFSKLAGKKLKNLLISG.NH_2) has the same antimicrobial activity as the intact peptide [58]. The fragment displayed potent and rapid bactericidal activity (MIC = 1 μM) against multidrug-resistant strains of *P. aeruginosa* and activity was partially preserved in the presence of 40% serum [59].

Peptides Active Against Polymicrobial Infections of Foot Ulcers in Diabetic Patients

Foot infections are the most common cause of hospitalisations and amputations in diabetic patients. They occur after skin ulcers or trauma in patients with peripheral neuropathy, sometimes together with vascular disease and are generally polymicrobial. Although the naturally occurring magainins from African clawed frogs of the genus *Xenopus*have only moderate potency against microorganisms, the analog-pexiganan acetate (MSI-78) showed distinct promise as a topical anti-infective agent for the treatment of infected foot ulcers in diabetic patients. Pexiganan represents a more cationic analogue of magainin-2 that contains an additional five lysine residues and an α-amidated C-terminus[60]. Pexiganan exhibited broad-spectrum antibacterial activity when tested against 3,109 clinical isolates of Gram-positive and Gram-negative aerobic and anaerobic bacteria [61]. The MIC value at which 90% of isolates were inhibited (MIC_{90}) was less than or equal to 32 μg/ml for all microorganisms tested except *Proteus mirabilis* and *Serratiamarcescens*. For 92% of the isolates tested, MBCs were the same or within a twofold difference of the MIC, consistent with a bactericidal action. A related study involving 2515 bacterial isolates from infected foot ulcers from diabetic patients produced similar results with MIC_{90} values for pexiganan of 16 μg/ml or less for Gram-positive aerobes, Gram-negative aerobes and facultative anaerobes[62]. It was concluded that pexiganan exhibits properties *in vitro* which make it an attractive candidate for development as a topical antimicrobial agent[63]. In phase III multicentre, randomised, double-blind trials in diabetic patients with infected foot ulcers, topical application of pexiganan acetate achieved clinical cure or improvement in about 90% of patients and the agent was well tolerated. However, the US Food and Drug Administration did not approve marketing of this peptide on the grounds that efficacy had not been sufficiently demonstrated.

Future Clinical Applications of Frog Skin Antimicrobial Peptides

The failure of pexiganan to become established in clinical practice resulted in a substantial decline in interest in frog skin antimicrobial peptides by the pharmaceutical industry. For progress in the field to continue, new clinical applications need to be found. Acne vulgaris is a disease of the pilosebaceous unit with both bacterial and inflammatory components. The Gram-positive anaerobic bacillus *Propioni bacterium acnes* is found in normal human cutaneous flora and colonisation and proliferation by this organism plays a major role in the development of an acne lesion[64]. Bacterial colonisation is preceded by hyperproliferation of keratinocytes and increased sebum secretion in a hair follicle together with stimulation of release of cytokines, such as interleukin (IL)-6 and IL-8 by follicular keratinocytes and IL-8 and IL-12 by macrophages[65]. Antibiotic resistance in *P. acnes* following prolonged monotherapy has been documented [66].

The acyclic brevinin-1 peptide RV-23 from *R. draytonii* (originally described as a melittin-related peptide)[19] showed potent growth-inhibitory activity (MIC < 10 μM) against isolates of *P. acnes* from blood cultures [67]. Previous studies have shown that cationic antimicrobial peptides, as well as possessing microbicidal actions, will inhibit the release of proinflammatory cytokines and so may reduce the inflammatory response that follows bacterial skin colonisation[68,69]. Thus, further studies are warranted to determine whether frog skin

peptides such as RV-23 may exercise a dual beneficial role in acne treatment by manifesting a bactericidal action on *P. acnes* and an anti-inflammatory effect on host cells.

In a similar manner, the formation of microbial biofilms in the oral cavity can initiate a cascade of inflammatory responses that lead to the destruction of gingival tissues and ultimately tooth loss. There is an extensive literature relating to antimicrobial peptides and proteins in saliva and gingival crevicular fluid that provide protection against pathogenic microorganisms[70]. Magainin and selected analogs show potent and rapid bactericidal activity against a range of anaerobic oral pathogens such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Prevotella* spp. [71]. More recently, caerulein precursor fragment CPF-AM1 from the African clawed frog *Xenopusamieti*[72] has shown particularly high potency (MIC < 2.5 μM) against the cariogenic microorganisms *Streptococcus mutans* and *Lactobacillus acidophilus* (F. Lundy and J.M. Conlon, unpublished data). Consequently, a role of such frog skin peptides in the prevention and treatment of periodontal disease is a possibility.

Acknowledgements

The experimental work carried out at U.A.E. University was supported by Faculty Support Grants and Interdisciplinary Grants from the institution and by the Terry Fox Fund for Cancer Research.

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