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Analogues of the frog skin peptide alyteserin-2a with enhanced antimicrobial activities against Gram-negative bacteria

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The emergence of strains of multidrug-resistant Gram-negative bacteria mandates a search for new types of antimicrobial agents. Alyteserin-2a (ILGKLLSTAAGLLSNL.NH_2) is a cationic, α-helical peptide, first isolated from skin secretions of the midwife toad, *Alytes obstetricans*, which displays relatively weak antimicrobial and haemolytic activities. Increasing the cationicity of alyteserin-2a while maintaining amphipathicity by the substitution $Gly^{11} \rightarrow$ Lys enhanced the potency against both Gram-negative and Gram-positive bacteria by between fourfold and 16-fold but concomitantly increased cytotoxic activity against human erythrocytes by sixfold (mean concentration of peptide producing 50% cell death; $LC_{50} = 24$ μM). Antimicrobial potency was increased further by the additional substitution $Ser^{7} \rightarrow$Lys, but the resulting analogue remained cytotoxic to erythrocytes ($LC_{50} = 38$ μM). However, the peptide containing D-lysine at positions 7 and 11 showed high potency against a range of Gram-negative bacteria, including multidrug-resistant strains of *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* (minimum inhibitory concentration = 8 μM) but appreciably lower haemolytic activity ($LC_{50} = 185$ μM) and cytotoxicity against A549 human alveolar basal epithelial cells ($LC_{50} = 65$ μM). The analogue shows potential for treatment of nosocomial pulmonary infections caused by bacteria that have developed resistance to commonly used antibiotics. Copyright © 2012 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: antimicrobial peptide; alyteserin-2a; structure–activity; Gram-negative bacteria

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 [1]. Although effective new types of antibiotics against multidrug-resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* 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]. Pulmonary infections, particularly nosocomial pneumonia, caused by these carbapenemase-producing bacteria that are resistant to all β-lactam antibiotics are particularly difficult to treat [3]. Colistin, the cyclic antibiotic peptide polymyxin E derived from *Bacillus polymyxa*, is used as a 'last resort' therapy in the management of infections because of multidrug-resistant Gram-negative bacteria [4]. However, not only high nephrotoxicity limits its use but also resistance to colistin has already been described, and increasing use of this antibiotic is likely to lead to the emergence of more resistant strains [5]. This situation has necessitated a search for novel types of antimicrobial agents, with appropriate toxicological and pharmacokinetic properties, to which the pathogenic microorganisms have not been exposed.

Skin secretions from Anura (frogs and toads) contain a wide range of compounds with biological activity that has excited interest because of their potential for drug development. Peptides with potent antimicrobial activity are present in skin secretions from many, but by no means all, frog species and are being increasingly considered as potential anti-infective agents [6]. These compounds play an important role in the system of innate immunity that predates adaptive immunity and constitutes the animal's first-line defense against invading pathogens [7,8]. Although there is no single mechanism by which the peptides produce cell death [9], their action generally does not involve binding to specific receptors on the cell membrane or to specific intracellular targets so that development of resistance to antimicrobial peptides takes place at rates that are orders of magnitude lower than those observed for conventional antibiotics [10].

Alyteserin-2a (ILGKLLSTAAGLLSNL.NH_2) was first isolated from norepinephrine-stimulated skin secretions from the midwife toad, *Alytes obstetricans* [11]. In common with most frog skin antimicrobial peptides [12], the peptide is cationic (molecular charge = +2 at pH 7), contains a high proportion of hydrophobic amino acids, and has the propensity to adopt an α-helical conformation in a membrane-mimetic solvent such as 50% TFE–water. Preliminary data indicate that alyteserin-2a, and the paralog

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alyteserin-2b, possess only weak growth-inhibitory activity against reference strains of Gram-negative and Gram-positive bacteria, and their haemolytic activities against human erythrocytes are relatively low ($LC_{50} > 100$ μM) [11]. The present structure–activity study investigates the effect of selective substitutions of amino acids in alyteserin-2a by either L-lysine or D-lysine on antimicrobial activity and on cytotoxicity against A549 cells and human erythrocytes. The A549 cell line is derived from a human lung adenocarcinoma and has been used extensively as a type II pulmonary epithelial cell model in drug metabolism and other studies [13]. The aim of the investigation was to develop a non-toxic peptide with high potency against clinically relevant microorganisms that has therapeutic potential as an anti-infective agent particularly for use against nosocomial pulmonary infections.

Materials and Methods

Peptide synthesis

Alyteserin-2a and its analogues, and alyteserin-2b were supplied in crude form by GL Biochem Ltd (Shanghai, China). The peptides were purified to near homogeneity using reversed phase HPLC on a (2.2 × 25-cm) Vydac 218TP1022 (C-18) column (Grace, Deerfield, IL, USA) equilibrated with acetonitrile/water/TFA (28.0/71.9/0.1, v/v/v) at a flow rate of 6 ml/min. The concentration of acetonitrile was raised to 56% (v/v) over 60 min using a linear gradient. Absorbance was measured at 214 and 280 nm, and the major peak in the chromatogram was collected manually. The final purity of all peptides tested was >98%. The purities and identities of the synthetic peptides were confirmed by electrospray mass spectrometry.

Microorganisms

Reference strains of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25726), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 90028) were purchased from the American Type Culture Collection (Rockville, MD, USA). *Acinetobacter baumannii* strain NM8 (clonal lineage Euroclone I) was isolated at a hospital in Abu Dhabi Emirate and was resistant to all antibiotics commonly used to treat *Acinetobacter* infections including cephalosporins, carbapenems, fluroquinolones, and aminoglycosides but remained sensitive to tigecycline and colistin [14]. *Stenotrophomonas maltophilia* B32/4 strain was a bloodstream isolate from a patient in an Abu Dhabi hospital and was resistant to meropenem but susceptible to cotrimoxazole [15]. The biofilm-producing *Staphylococcus epidermidis* RP62A strain produces polysaccharide intercellular adhesin that protects the bacteria against the components of the human innate immune system. Its full genome sequence is in the GenBank: NC002976. *S. epidermidis* RP62A/1 is a stable biofilm non-producer phase variant of RP62A [16].

Antimicrobial assays

Minimum inhibitory concentration (MIC) of the peptides was determined in duplicate in three independent experiments using standard microdilution methods using 96-well microtiter cell-culture plates [17,18]. Serial dilutions of the peptides in Mueller-Hinton broth (50 μl) were mixed with an inoculum (50 μl of 10^6 colony forming units (CFU/ml) from a log-phase culture. The initial inoculum of each microorganism was prepared as previously described [19]. Bacteria were incubated for 18 h at 37 °C in a humidified atmosphere of air. *C. albicans* was incubated in RPMI 1640 medium for 48 h at 35 °C, with an inoculum of 5×10^4 CFU/ml. After incubation, MIC was taken as the lowest concentration of peptide where no visible growth was observed. This value was confirmed by determining the absorbance at 630 nm of each well using a microtiter plate reader. In order to monitor the validity and reproducibility of the assays, incubations with bacteria were carried out in parallel with increasing concentrations of ampicillin or ciprofloxacin and incubations with *C. albicans* in parallel with amphotericin B.

Cytotoxicity assays

Peptides in the concentration range 16–500 μM were incubated with washed human erythrocytes (2×10^7 cells) from a healthy donor in Dulbecco's phosphate-buffered saline, pH 7.4 (100 μl) for 1 h at 37 C. After centrifugation (12 000*g* for 15 s), the absorbance at 450 nm of the supernatant was measured. A parallel incubation in the presence of 1% v/v Tween-20 was carried out to determine the absorbance associated with 100% haemolysis. The LC_{50} value was taken as the mean concentration of peptide producing 50% haemolysis in three independent experiments.

Human A549 alveolar basal epithelial cells were maintained at 37 C in RPMI 1640 medium containing 2 mM L-glutamine and supplemented with 10% foetal calf serum (FBS, Biowest, Nouaille, France), and antibiotics (penicillin 50 U/ml; streptomycin 50 μg/ml). Cell viability was higher than 99% using trypan blue dye exclusion in all experiments. Cells were seeded in 96-well plates at a density of 5×10^3 cells/well. After 24 h, cells were treated for 24 h with increasing concentrations of alyteserin-2a and its analogues (1–100 μM) in triplicate. Control cultures were treated with medium only. The effect of the peptides on cell viability was determined by measurement of ATP concentrations using a CellTiter-Glo Luminescent Cell Viability assay (Promega Corporation, Madison, WI, USA). Luminescent signals were measured using a GLOMAX Luminometer system. The LC_{50} value was taken as the mean concentration of peptide producing 50% cell death in three independent experiments.

Estimation of effective hydrophobicity

Peptides (approximately 5 nmol in 200 μl) were injected onto a (25 × 0.46-cm) Vydac 218TP54 (C-18) column equilibrated with acetonitrile / water/trifluoroacetic acid (21.0/78.9/0.1) at a flow rate of 1.5 ml/min. The concentration of acetonitrile was raised to 49.0% over 40 min using a linear gradient. Absorbance was measured at 214 nm, and the retention time of each peptide was recorded.

Secondary structure prediction

Prediction of secondary structure and determination of helicity per residue for the peptides were performed using the AGADIR programme [20]. AGADIR is a prediction algorithm based on the helix/coil transition theory that predicts the helical behaviour of monomeric peptides. Calculations were performed at pH 7, ionic strength 0.1 M, and 278 °K. A minimum percentage of 1% helicity/residue was considered to predict the presence of a helix.

Results

Physicochemical properties of the peptides

The primary structures, molecular charge at pH 7, retention time on reversed phase HPLC, calculated mean hydrophobicities, and

predicted helicity using the AGADIR programme of the peptides investigated in this study are shown in Table 1. Retention time on reversed phase HPLC has been shown to provide a measure of the effective hydrophobicity of an α-helical peptide [21]. As shown in Table 1, small changes in structure (substitution of single amino acid residues) produced appreciable changes in the effective hydrophobicity of the peptides as indicated by their chromatographic properties. As expected, the retention times of the analogues [G3K], [S7k] and [S7k, S11k] were less than that of alyteserin-2a consistent with the greater hydrophobicity of glycine and serine relative to lysine [22]. However, the appreciably increased retention times of the [S7K], [G11K], and [N15K] do not correlate with calculated hydrophobicity. Analysis of the secondary structure of the peptides using the AGADIR programme [20] indicates that alyteserin-2a has the propensity to adopt an α-helical conformation between residues 9–14 (maximum percentage helicity/residue = 1.5), whereas alyteserin-2b has a low probability of adopting a helical conformation (maximum percentage helicity/residue = 0.8). The substitution $Ser^{7}\rightarrow$L-Lys in alyteserin-2a does not stabilise the helix (maximum percentage helicity/residue = 1.4). However, the substitution $Asn^{15}\rightarrow$L-Lys promotes the stability of the helical conformation (maximum percentage helicity/residue = 2.6) and the substitution $Gly^{11}\rightarrow$L-Lys has a pronounced effect on helix stability (maximum percentage helicity/residue = 8.2). A Schiffer–Edmundson wheel representation [23] of alyteserin-2a illustrates the amphipathic nature of the α-helical conformation with the hydrophilic Lys^{4}, Ser^{7}, and Ser^{14} segregating on one face of the helix and the hydrophobic leucine residues on the opposite face (Figure 1).

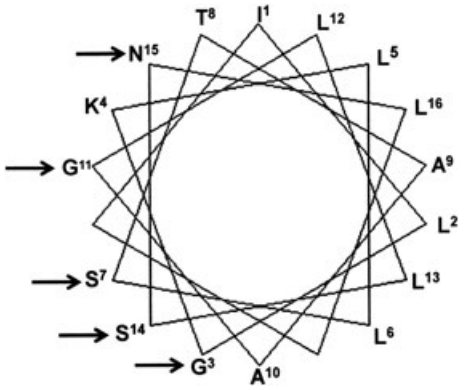

Figure 1. Schiffer–Edmundson wheel representation of alyteserin-2a demonstrating the amphipathic nature of the α-helical conformation. The arrows denote the sites of replacement of amino acids by lysine in order to produce analogues with greater antimicrobial potencies.

Antimicrobial and cytotoxic activities of the peptides

The abilities of synthetic alyteserin-2a and alyteserin-2b to inhibit the growth of reference strains and antibiotic-resistant clinical isolates of bacteria and the opportunistic yeast pathogen *C. albicans* are compared in Table 2. Neither peptide shows high potency (MIC ≥ 32 μM), but the activity of alyteserin-2a was greater against all microorganisms tested and so this peptide was selected for structure–activity studies.

Substitution of the amino acid residues on the hydrophilic face of alyteserin-2a (Gly^{3}, Ser^{7}, Gly^{11}, Ser^{14}, and Asn^{15}) by L-Lys results in increased potency against several Gram-negative and Gram-positive bacteria and against the opportunistic yeast pathogen *C. albicans* (Table 2). Overall, the [G11K] analogue is the most active of the monosubstituted peptides against all microorganisms tested. However, the haemolytic activities of all the L-Lys-containing analogues are greater than the naturally occurring peptide. The [G11K] analogue, as well as being the most potent against microorganisms, is the most haemolytic (LC_{50} = 24 μM). Increasing cationicity further by substitution of both Ser^{7} and Gly^{11} on the hydrophilic face of the helix by L-Lysine results in increased antimicrobial potency against all microorganisms so that the [S7K,G11K] analogue is now 32-fold more potent than the native peptide against *S. maltophilia* (MIC = 4 μM) and the biofilm-producing strain of *S. epidermidis* (MIC = 2 μM) (Table 2). However, this peptide retains high haemolytic activity (LC_{50} = 38 μM).

The attempt to increase cationicity while simultaneously reducing the degree of helicity of alyteserin-2a by substitution of Ser^{7} by D-lysine has a deleterious effect on antimicrobial potency against Gram-positive bacteria and does not appreciably increase potency against Gram-negative bacteria. The analogue is still more haemolytic than the naturally occurring peptide (LC_{50} = 105 μM) (Table 2). In contrast, [G11k]alyteserin-2a and the disubstituted analogue [S7k,G11k]alyteserin-2a retain relatively high antimicrobial potency against both Gram-negative and

Table 1. Physicochemical properties of alyteserin-2a and its analogues

Peptide	Amino acid sequence	Charge	RT (min)	GRAVY	Helical domain
Alyteserin-2a	ILGKLLSTAAGLLSNL.NH_2	+2	28.0	1.275	9-14
Alyteserin-2b	ILGAILPLVSGLLSNKL.NH_2	+2	28.7	1.553	Non-helical
[G3K]	ILKKLLSTAAGLLSNL.NH_2	+3	24.9	1.056	9-14
[S7K]	ILGKLLKTAAGLLSNL.NH_2	+3	37.3	1.081	9-14
[G11K]	ILGKLLSTAAKLLSNL.NH_2	+3	31.8	1.056	7-16
[S14K]	ILGKLLSTAAGLLKNL.NH_2	+3	28.5	1.081	8-16
[N15K]	ILGKLLSTAAGLLSKL.NH_2	+3	41.2	1.250	8-16
[S7K,G11K]	ILGKLLKTAAKLLSNL.NH_2	+4	30.3	0.863	4-16
[S7k]	ILGKLLkTAAGLLSNL.NH_2	+3	19.5	1.081	ND
[G11k]	ILGKLLSTAAkLLSNL.NH_2	+3	31.9	1.056	ND
[S7k,G11k]	ILGKLLkTAAkLLSNL.NH_2	+4	22.3	0.863	ND

Retention time (RT) was measured on a Vydac 218TP54 column using the elution conditions described in the text. Grand average of hydropathicity (GRAVY) of the peptides is calculated using the hydrophobicity scales for amino acid residues of Kyte and Doolittle [21]. The symbol k represents D-Lys. ND indicates not determined.

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Table 2. Minimum inhibitory concentrations of alyteserin-2a and its analogues against microorganisms and cytotoxicities (LC_{50} values) against human erythrocytes and A549 human pulmonary epithelial cells

	Aly-2a	Aly-2b	G3KKK	S7K	G11K	S14K	N15K	S7K G11K	S7k	G11k	S7k G11k
S. aureus ATCC 25923	64	128	64	8	4	16	32	4	>128	4	64
S. epidermidis RP62A/1	32	128	16	4	4	8	8	2	128	4	8
S. epidermidis RP62A	64	128	32	4	4	16	16	2	128	4	8
E. coli ATCC 25726	256	>256	32	64	32	32	>256	16	128	32	16
P. aeruginosa ATCC 27853	256	>256	128	64	32	128	>128	8	>128	32	32
K. pneumoniae ATCC 700603	256	>256	128	64	32	128	>128	16	>128	64	32
A. baumannii NM8	64	128	16	64	8	32	>128	4	64	8	8
S. maltophilia B32/4	128	256	32	64	32	64	>128	4	128	16	8
C. albicans ATCC 90028	64	64	32	128	16	32	>128	8	64	16	8
Erythrocytes	140	115	105	28	24	55	50	38	105	42	185
A549 cells	80	>100	100	35	20	30	13	20	>100	28	65

Data are expressed in μM.

Gram-positive bacteria especially clinical isolates of multidrug-resistant *A. baumannii* and *S. maltophilia* and a biofilm-producing strain of *S. epidermidis*. The peptide also inhibited the growth of reference strains of *P. aeruginosa* and *K. pneumomiae* (MIC = 32 μM). [G11k]alyteserin-2a shows high haemolytic activity (LC_{50} = 42 μM), whereas [S7k,G11k]alyteserin-2a is less haemolytic than alyteserin-2a (LC_{50} = 185 μM).

Alyteserin-2a (LC_{50} = 80 μM) and alyteserin-2b (LC_{50} > 100 μM) show relatively weak cytotoxic activity against A549 human alveolar epithelial cells (Figure 2). The twofold to fourfold increase in cytotoxicity of the [S7K], [G11K], [S14K], [S7K,G11K], and [G11k] analogues is mirrored by a comparable increase in haemolytic activity against human erythrocytes (Table 2). The substitution $Asn^{15}\rightarrow$L-Lys produces the greatest (sixfold) increase in cytotoxicity against the A549 cells (Figure ure 2) and the [N15K] analogue is fourfold more potent against A549 cells than erythrocytes. Among all the analogues synthesised, [S7k,G11k] alyteserin-2a exhibits the best antibacterial activity *versus* cytotoxicity ratio. The therapeutic index of this peptide, defined as the ratio of LC_{50} to MIC, for *A. baumannii*, *S. maltophilia*, *S. epidermidis*, and *C. albicans* is 23 *versus* erythrocytes and 8 *versus* A549 cells.

Discussion

The relative potencies of cell-penetrating peptides against bacteria and fungi and against mammalian cells are determined by complex interactions between cationicity, hydrophobicity, conformation (α-helicity), and amphipathicity [24,25]. These parameters are not independent variables so that alteration of peptide structure by a selected amino acid substitution may change several physicochemical properties simultaneously. The bacterial cell membrane is associated with a greater negative charge than the plasma membrane of mammalian cells because of a higher proportion of anionic phospholipids so that an increase in peptide cationicity, while maintaining amphipathicity, should enhance antimicrobial potency without increasing toxicity against mammalian cells. Studies with the naturally occurring amphipathic α-helical peptides magainin-2 [26,27] and pseudin-2 [28] have demonstrated that increasing the positive charge on the peptides does produce an increase in antimicrobial activity until a limit is reached, whereupon further increase in cationicity does not result in any further increase in activity. Consistent with data obtained with analogues of the frog skin antimicrobial peptides, alyteserin-1c [29], ascaphin-8 [12], B2-RP [14], kassinatuerin-1 [30], peptide XT-7 [12], and temporin-DRa [31], increasing the cationicity of alyteserin-2a while maintaining amphipathicity of the helical conformation by appropriate substitutions by L-lysine results in increased potency against both Gram-negative and Gram-positive bacteria and against the opportunistic yeast pathogen *C. albicans* (Table 2). However, except for the [G3K] analogue, this increased antimicrobial activity was associated with appreciably increased cytotoxicity against erythrocytes and A549 cells.

Studies with a range of naturally occurring [28,32–34] and model amphipathic α-helical peptides [35–37] have shown that increasing mean hydrophobicity generally results in an increase in cytotoxicity against erythrocytes and other mammalian cells thereby decreasing the specificity of the peptide for microorganisms. In the case of analogues of the 26-residue amphipathic α-helical antimicrobial peptide V13KL, data indicate that there is an optimum range of hydrophobicities outside of which antimicrobial activity dramatically decreases. Loss of antimicrobial activity at high peptide hydrophobicity can be explained by the strong peptide self-association that prevents the peptide from passing through the cell wall in prokaryotic cells, whereas increased peptide self-association had no effect on peptide access to eukaryotic membranes [37]. Consistent with previous studies with magainin-2 [21] and temporin-DRa [31] analogues, the hydrophobicity of the lysine-substituted analogues of alyteserin-2a could not be accurately described by a simple summation of the hydrophobicities of each constituent amino acid (Table 1). However, the observed hydrophobicity reflected in the retention time on reversed phase HPLC does correlate moderately well with the observed cytotoxic activities of the peptides against mammalian cells. Thus, the [N15K] analogue has the highest retention time and shows the greatest activity against A549 cells, whereas the [S7k] and [G3K] peptides have the lowest retention times of the monosubstituted peptides and the least cytotoxic activities. Increases in the degree of α-helicity of a cell-penetrating peptide also increase cytolytic activity against mammalian cells [38] so that in the case of the [N15K] and [G11K] analogues, the increased stability of the α-helical conformation will contribute to the observed increased activities

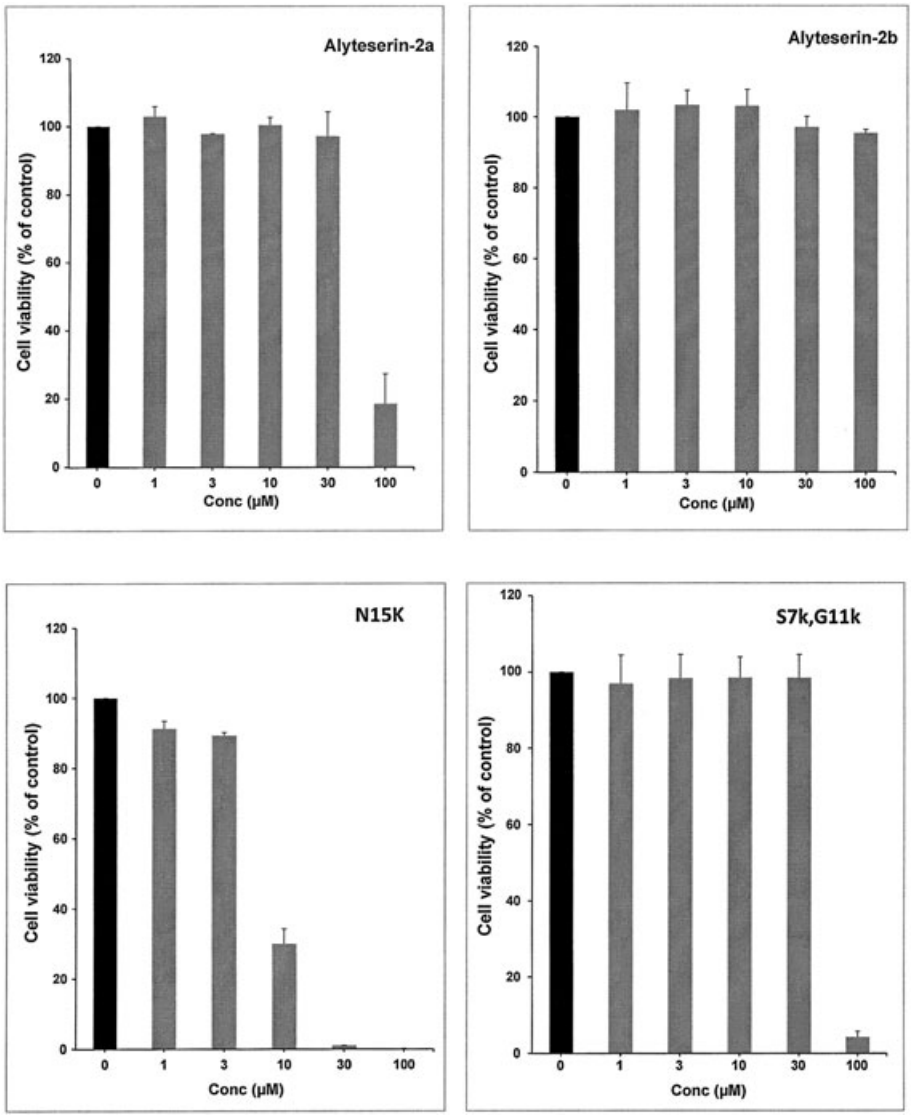

Figure 2. Effects of alyteserin-2a, alytesterin-2b, [N15K]alyteserin-2a, and [S7k, G11k] alyteserin-2a on the viability of A549 pulmonary epithelial cells after 24-h exposure. All experiments were repeated at least three times. Columns indicate mean; bars, SEM.

against erythrocytes and A549 cells. Conversely, the reduced haemolytic activity of the [S7k,G11k] analogue is probably a consequence of reduced helicity produced by the helix-destabilising D-lysine residues as well as reduced hydrophobicity.

Nosocomial pneumonia (including ventilator-associated pneumonia) is one of the most common infectious complications in the intensive care unit (ICU) and is a leading cause of death [39]. Treatment options are severely limited when infection is caused by multidrug-resistant or pandrug-resistant bacteria [40]. Aerosolised colistin has been used with some success as an adjunct to intravenous therapy in ICU patients infected with antibiotic-resistant strains of *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* [41,42]. Intranasal colistin has also been used to treat infections in patients with cystic fibrosis [43]. This study has demonstrated that analogues of alyteserin-2a, particularly the [S7k,G11k] derivative, show potent growth-inhibitory activity against a range of Gram-negative opportunistic pathogens including clinical isolates of multidrug-resistant strains of *A. baumannii* and *S. maltophilia* and reference strains of *P. aeruginosa*, and *K. pneumoniae*. This activity is combined with appreciably lower toxicity against human erythrocytes and A549 human alveolar basal epithelial cells and good solubility at physiological pH. Compared with antimicrobial peptides in current clinical trials such as Omiganan [44], the therapeutic index of [S7k,G11k]alyteserin-2a is probably too low for systemic use, but the peptide shows promise as a starting compound for development into drug that can be administered intranasally for treatment of pulmonary infections caused by pathogenic bacteria that have developed resistance to commonly used antibiotics.

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References

1 Livermore DM. Has the era of untreatable infections arrived? *J. Antimicrob. Chemother.* 2009; **64 Suppl 1**: i29–36.
2 Giamarellou H, Poulakou G. Multidrug-resistant Gram-negative infections: what are the treatment options? *Drugs* 2009; **69**: 1879–1901.
3 Kollef MH. Review of recent clinical trials of hospital-acquired pneumonia and ventilator-associated pneumonia: a perspective from academia. *Clin. Infect. Dis.* 2010; **51(**Suppl 1**)**: S29–35.
4 Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski A 3rd, Forrest A, Bulitta JB, Tsuji BT. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. *Pharmacotherapy* 2010; **30**: 1279–1291.
5 Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, Liolios L. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 2006; **50**: 2946–2950.
6 Conlon JM, Sonnevend A. Clinical applications of amphibian antimicrobial peptides. *J. Med. Sci.* 2011; **4**: 62–72.
7 Conlon JM. The contribution of skin antimicrobial peptides to the system of innate immunity in anurans. *Cell Tissue Res.* 2011; **343**: 201–212.
8 Diamond G, Beckloff N, Weinberg A, Kisich KO. The roles of antimicrobial peptides in innate host defense. *Curr. Pharm. Des.* 2009; **15**: 2377–2392.
9 Bocchinfuso G, Palleschi A, Orioni B, Grande G, Formaggio F, Toniolo C, Park Y, Hahm KS, Stella L. Different mechanisms of action of antimicrobial peptides: insights from fluorescence spectroscopy experiments and molecular dynamics simulations. *J. Pept. Sci.* 2009; **15**: 550–558.
10 Perron GG, Zasloff M, Bell G. Experimental evolution of resistance to an antimicrobial peptide. *Proc. Biol. Sci.* 2006; **273**: 251–256.
11 Conlon JM, Demandt A, Nielsen PF, Leprince J, Vaudry H, Woodhams DC. The alyteserins: two families of antimicrobial peptides from the skin secretions of the midwife toad *Alytes obstetricans* (Alytidae). *Peptides* 2009; **30**: 1069–1073.
12 Conlon JM. Structural diversity and species distribution of host-defense peptides in frog skin secretions. *Cell. Mol. Life Sci.* 2011; **68**: 2303–2315.
13 Foster KA, Oster CG, Mayer MM, Avery ML, Audus KL. Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. *Exp. Cell Res.* 1998; **243**: 359–366.
14 Conlon JM, Ahmed E, Condamine E. Antimicrobial properties of brevinin-2-related peptide and its analogs: efficacy against multidrug-resistant *Acinetobacter baumannii*. *Chem. Biol. Drug Des.* 2009; **74**: 488–493.
15 Jumaa PA, Sonnevend A, Pàl T, El Hag M, Amith R, Trad O. The molecular epidemiology of *Stenotrophomonas maltophilia* bacteraemia in a tertiary referral hospital in the United Arab Emirates 2000–2004. *Ann. Clin. Microbiol. Antimicrob.* 2006; **5**: 32.
16 Ziebuhr W, Krimmer V, Rachid S, Lössner I, Götz F, Hacker J. A novel mechanism of phase variation of virulence in *Staphylococcus epidermidis*: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. *Mol. Microbiol.* 1999; **32**: 345–356.
17 Clinical Laboratory and Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M07-A8.CLSI, Wayne PA, 2008.
18 Clinical Laboratory and Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeast. Approved Standard M27-A3. CLS1, Wayne, PA, 2008.
19 Conlon JM, Sonnevend A. Antimicrobial peptides in frog skin secretions. *Methods Mol. Biol.* 2010; **618**: 3–14.
20 Muñoz V, Serrano L. Elucidating the folding problem of helical peptides using empirical parameters. *Nature Struct. Biol.* 1994; **1**: 399–409.
21 Tachi T, Epand RF, Epand RM, Matsuzaki K. Position dependent hydrophobicity of the antimicrobial magainin peptide affects the mode of peptide-lipid interactions and selective toxicity. *Biochemistry* 2002; **41**: 10723–10731.
22 Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 1982; **157**: 105–132.
23 Schiffer M, Edmundson AB. Use of helical wheels to represent the structures of proteins and to identify segments with helical potential. *Biophys. J.* 1967; **7**: 121–135.
24 Dathe M, Wieprecht T. Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells. *Biochim. Biophys. Acta* 1999; **1462**: 71–87.
25 Kindrachuk J, Napper S. Structure-activity relationships of multifunctional host defense peptides. *Mini Rev. Med. Chem.* 2010; **10**: 596–614.
26 Bessalle R, Haas H, Goria A, Shalit I, Fridkin M. Augmentation of the antibacterial activity of magainin by positive-charge chain extension. *Antimicrob. Agents Chemother.* 1992; **36**: 313–317.
27 Gottler LM, Ramamoorthy A. Structure, membrane orientation, mechanism, and function of pexiganan—a highly potent antimicrobial peptide designed from magainin. *Biochim. Biophys. Acta* 2009; **1788**: 1680–1686.
28 Pál T, Sonnevend A, Galadari S, Conlon JM. Design of potent, non-toxic antimicrobial agents based upon the structure of the frog skin peptide, pseudin-2. *Regul. Pept.* 2005; **129**: 85–91.
29 Conlon JM, Ahmed E, Pal T, Sonnevend A. Potent and rapid bactericidal action of alyteserin-1c and its [E4K] analog against multidrug-resistant strains of *Acinetobacter baumannii*. *Peptides* 2010; **31**: 1806–1810.
30 Conlon JM, Abraham B, Galadari S, Knoop FC, Sonnevend A, Pál, T. Antimicrobial and cytolytic properties of the frog skin peptide, kassinatuerin-1 and its L- and D-lysine-substituted derivatives. *Peptides* 2005; **26**: 2104–2110.
31 Conlon JM, Al-Ghaferi N, Abraham B, Leprince J. Strategies for transformation of naturally-occurring amphibian antimicrobial peptides into therapeutically valuable anti-infective agents. *Methods* 2007; **42**: 349–357.
32 Maloy WL, Kari UP. Structure-activity studies on magainins and other host defense peptides. *Biopolymers* 1995; **37**: 105–122.
33 Dathe M, Wieprecht T, Nikolenko H, Handel L, Maloy WL, MacDonald DL, Beyermann M, Bienert M. Hydrophobicity, hydrophobic moment and angle subtended by charged residues modulate antibacterial and haemolytic activity of amphipathic helical peptides. *FEBS Lett.* 1997; **403**: 208–212.
34 Wieprecht T, Dathe M, Beyermann M, Krause E, Maloy WL, MacDonald DL, Bienert M. Peptide hydrophobicity controls the activity and selectivity of magainin 2 amide in interaction with membranes. *Biochemistry* 1997; **36**: 6124–6132.
35 Stark M, Liu LP, Deber CM. Cationic hydrophobic peptides with antimicrobial activity. *Antimicrob. Agents Chemother.* 2002; **46**: 3585–3590.
36 Wang P, Nan YH, Yang ST, Kang SW, Kim Y, Park IS, Hahm KS, Shin SY. Cell selectivity and anti-inflammatory activity of a Leu/Lys-rich alpha-helical model antimicrobial peptide and its diastereomeric peptides. *Peptides* 2010; **31**: 1251–1261.
37 Chen Y, Guarnieri MT, Vasil AI, Vasil ML, Mant CT, Hodges RS. Role of peptide hydrophobicity in the mechanism of action of alpha-helical antimicrobial peptides. *Antimicrob. Agents Chemother.* 2007; **51**: 1398–406.
38 Dathe M, Schümann M, Wieprecht T, Winkler A, Beyermann M, Krause E, Matsuzaki K, Murase O, Bienert M. Peptide helicity and membrane surface charge modulate the balance of electrostatic and hydrophobic interactions with lipid bilayers and biological membranes. *Biochemistry* 1996; **35**: 12612–12622.
39 Wahl WL, Zalewski C, Hemmila MR. Pneumonia in the surgical intensive care unit: is every one preventable? *Surgery* 2011; **150**: 665–672.
40 Jean SS, Hsueh PR. Current review of antimicrobial treatment of nosocomial pneumonia caused by multidrug-resistant pathogens. *Expert Opin. Pharmacother.* 2011; **12**: 2145–2148.
41 Kwa AL, Loh C, Low JG, Kurup A, Tam VH. Nebulized colistin in the treatment of pneumonia due to multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Clin. Infect. Dis.* 2005; **41**: 754–757.
42 Michalopoulos A, Fotakis D, Virtzili S, Vletsas C, Raftopoulou S, Mastora Z, Falagas ME. Aerosolized colistin as adjunctive treatment of ventilator-associated pneumonia due to multidrug-resistant Gram-negative bacteria: a prospective study. *Respir. Med.* 2008; **102**: 407–412.
43 Beringer P. The clinical use of colistin in patients with cystic fibrosis. *Curr. Opin. Pulm. Med.* 2001; **7**: 434–440.
44 Melo MN, Dugourd D, Castanho MA. Omiganan pentahydrochloride in the front line of clinical applications of antimicrobial peptides. *Recent Pat. Antiinfect. Drug Discov.* 2006: **1**: 201–207.

