Poster No. F-256

Novel Arenicin-3 Peptide Antibiotics with Broad-spectrum Activity Against MDR Gram-negative Bacterial Act Via Dual Mode of Actions

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Introduction

Arenicin-3 (NZ17000) is a novel antimicrobial peptide isolated from the lugworm *Arenicola marina*. It is composed of 21 amino acids (GFCWYVCYRNGVRVC YRRCN), with two disulphide bonds bridging between Cys2, Cys20 and Cys7, Cys16. A series of arenicin-3 analogues were designed and synthesized to investigate the mode of action. These peptides exhibit potent, rapid antimicrobial activity *in vitro* against a broad range of multi-resistant pathogenic Gram-negative bacteria, including polymyxin resistant *Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae*. The peptides also demonstrate *in vivo* activity in urinary tract infection (UTI) and pneumonia mouse models. However, the mode of action of arenicin-3 remains elusive. Here, we employed a set of biological assays as well as genomic sequencing, Transposon Directed Insertion Sequencing (TraDIS) and molecular modeling approaches to elucidate the mode of action of this promising drug target.

Methods

Antimicrobial activity (MIC) was determined using published standard protocols (Wiegand et al., 2008). Membrane perturbation was assessed using dye-based diSC3-5 and NPN uptake assays. Morphological changes were confirmed by transmission electron microscopy (TEM) using a JEOL 1011 TEM system. SPR was performed using a BiaCore T200 system. Genomic sequencing were performed at the Queensland Centre for Medical Genomics (University of Queensland, Australia) on an Illumina MiSeq (Illumina, San Diego, CA). TraDIS methods were described in Poster C-1424. The modeling simulations were calculated using GROMACS (4.6) with a united-atom force field (54a7) and performed using pre-equilibrated lipid bilayers with explicit water and ions in a rectangular box.

Conclusion

Our results strongly suggest a dual mode of action of arenicin-3 against Gram-negative bacteria. Arenicin-3 binds to and disrupts the integrity of both outer and cytoplasmic membranes of Gram-negative bacteria through direct binding to phospholipids, independent of lipid A. TraDIS data suggested arenicin may interrupt phospholipid transportation pathways between the two membranes, leading to dis-regulation of membrane composition and compromised membrane integrity.

Activities

Compound ID	EC	KP	AB	PA	Cytotox	Haemolysis
	MIC [μg/mL]				CC^{50} [μM]	at 300 μg/mL
Colistin	≤0.03	≤0.03	0.06	0.25	>100	0%
Arenicin-3	1	2	0.5	2	30	10%
NZ17125	0.125	0.5	0.25	0.25	31	8%
NZ17126	0.125	0.25	0.25	0.25	77	0%
NZ17139	0.06	0.25	0.125	0.5	>100	0%
NZ17143	0.25	1	2	4	>100	0%
NZ17160	0.125	0.5	0.5	0.5	>100	0%
NZ17211	0.5	2	2	2	>100	0%
NZ17224	0.06	0.25	0.25	0.25	82	0%
NZ17228	0.25	0.25	0.25	0.5	>100	0%
NZ17230	0.125	0.25	0.25	0.25	100	0%

Table 1. Antimicrobial activity and cytotoxicity of arenicin-3 and its analogues. EC: *Escherichia coli* (ATCC 25922), KP: *K. pneumoniae* (ATCC13883), AB: *A. baumannii* (ATCC19606), PA: *P. aeruginosa* (ATCC27853), PAR: *P. aeruginosa* polymyxin resisntant strain (FADDI-PA070). Cytotoxicity and haemolysis assays were performed using HEK293 cells and human red blood cells, respectively.

Compound ID	PA1	PA2	AB1	AB2	KP1	KP2	KP3	KP4	KP5	KP6
	MIC [μg/mL]									
Colistin	>32	>32	>32	>32	32	>32	>32	>32	>32	>32
Arenicin-3	4	16	1	0.5	16	32	64	16	16	64
NZ17139	2	16	0.5	0.25	4	8	16	8	4	16
NZ17230	2	8	0.25	0.5	4	8	16	4	8	16
NZ17211	64	32	2	4	16	32	>64	32	32	64
NZ17143	8	32	2	1	8	16	32	16	16	64

Table 2. Antimicrobial activity against *A. baumannii* strains and clinical resistant strains. PA: *P. aeruginosa* (PA1, FADDI-PA070; PA2, polymyxin resistant strain), AB1-2: *A. baumannii,* KP1-6: *K. pneumonia* clinical isolates.

Modeling

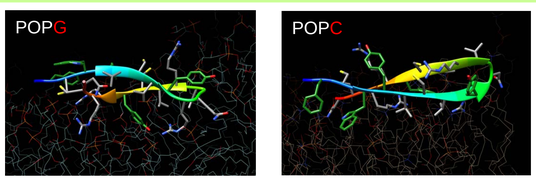

Figure 1. Molecular dynamics simulation of arenicin-3 with POPG and POPC lipid bilayers, representing a bacteria and mammalian type membrane respectively. Simulation show different orientation of the peptide with different interactions, illustrated by Trp^4 in the yellow circle. Simulation with POPG also show tighter interactions.

Mode of Actions

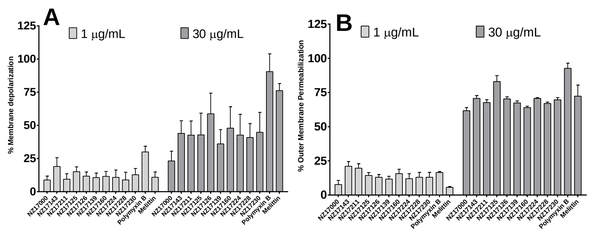

Figure 2. Membrane perturbation assays. (A) 1-N-phenylnaphthylamine (NPN) uptake assay showed the outer membrane pemeabilization of arenicin-3 and its analogues against *E.coli* cells. (B) DiSC3-5 assay indicated the inner membrane depolarization of *E.coli* cells when treated with arenicin-3 peptides. Polymyxin B and melittin were used as controls.

Figure 3. SPR assay of the binding of arenicin and its analogues to DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) and DMPC with 10% (mol/mol) lipid A. The binding of arenicin-3 and its analogues to membrane is lipid A-independent. (A) Binding at 1 μM; (B) Binding at 10 μM. Colistin and polymyxin B were used as controls.

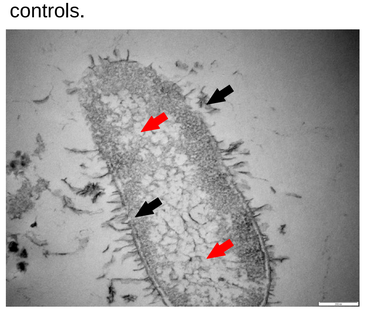

Figure 4. Transmission electron microscopy (TEM) of *P. aeruginosa* incubated with arenicin-3. Black arrows show the membrane disruption; red arrows indicate the low density of cytoplasm due to release. Scale Bar, 200 nm.

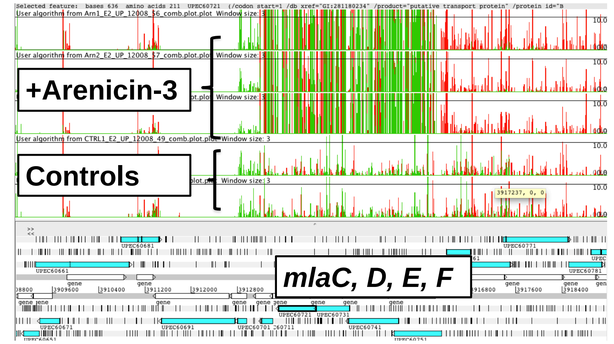

Figure 5. Genomic sequencing and TraDIS showed *mlaC* SNP provides arenicin resistance in *E. coli* UPEC strain. Clear increased insertion levels in *mlaC* (an increased mutant population) were observed in *E. coli* (whole operon). In addition, the whole *mla* operon is involved in sensitivity, not just *mlaC*.

The arenicin treated library is represented by the top 3 rows and the controls were indicated by the bottom 2 rows. *MlaC* genome is highlighted in black. Further details are shown in Poster No. C-1424

Acknowledgement

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