Biomimetic liposomes as delivery systems for antimicrobial peptides

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Introduction

700,000 deaths worldwide per year are caused by antimicrobial-resistant (AMR) infections.

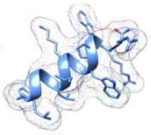
Zero classes of antibiotics have been discovered and are available for routine treatment since the 1980s.

Urgent need for new approaches to address AMR

One potential solution:
Liposomes represent one of the most widely used antimicrobial drug delivery nanosystems [1]. They have been proven to increase the bioavailability, biocompatibility and safety profiles of encapsulated drug.

Strategy

Antimicrobial peptides (AMPs), such as RN7IN6 [2] are promising, broad-spectrum anti-infectives that may provide alternatives to conventional antibiotics.



Biomimetic nanocarriers, such as liposomes composed of cell membrane-relevant phospholipids [3] (Fig. 1) are considered as innovative strategies for delivering antimicrobial agents.

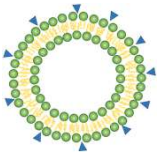


Fig. 1: RN7IN6 adsorbed biomimetic liposome.

The **aim** of this study was to prepare liposomes composed of bacteria membrane-relevant phospholipids and to synthesise and investigate the antimicrobial activity of a ranalexin analog, RN7IN6, for subsequent adsorption to biomimetic liposomes.

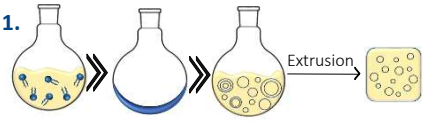
Methods

Peptide Synthesis

RN7IN6 was prepared via automated Fmoc-SPPS on a Liberty Blue automated peptide synthesiser. The peptide was characterised using LC-MS and RP-HPLC and purified using prep-HPLC.

Biomimetic liposome preparation

1.



Liposomes (POPE, POPG, CL at 70:20:10 weight ratio) were manufactured using the thin film hydration method followed by extrusion and characterised using a ZetasizerNano ZS .

2.

Preliminary biomimetic liposome (LP) formulations were also prepared via microfluidic mixing techniques. The impact of solvent selection was tested using 1-propanol and 1-propanol:DMF (80:20 w/w) to dissolve lipids. Different buffer:lipid flow rate ratios (FRR) were also investigated.

Antibacterial efficacy tests

Antimicrobial activity (MIC) of free RN7IN6 against wild-type *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. aureus* was tested, using a broth microdilution and resazuring dye as a cell viability marker. MBC (minimum bactericidal concentration) was measured by subculturing the broths used for MIC determination onto fresh agar plates.

Results

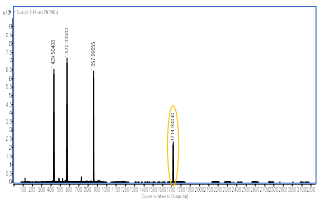


Fig. 2: RN7IN6 mass spectrum expressed as a mass to charge ratio [M+H]+.

RN7IN6 was successfully synthesised, characterised and purified at approximately 90% purity (Fig. 2).

Table 1: Size, PDI and Zeta Potential of empty and RN7IN6 adsorbed liposomes prepared using thin film hydration method

	Empty liposomes	RN7IN6 liposomes
Size (nm)	143.2 ± 0.46	175.7 ± 2.54
PDI	0.185 ± 0.003	0.358 ± 0.008
Zeta Potential (mV)	-30.9 ± 0.361	-19.3 ± 0.58

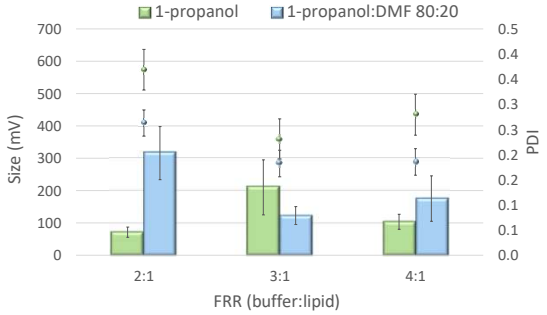


Fig. 3: Size and PDI of biomimetic liposomes prepared via the Nanoassemblr benchtop, using different solvents and FRR.

The surface charge of thin film hydration-produced liposomes decreased in magnitude following incubation with RN7IN6, indicative of successful peptide adsorption (Table 1). Microfluidic-manufactured liposomes produced using FRR 3:1 and 1-propanol:DMF as solvent (Fig.3) were comparable in size and PDI to liposomes prepared by thin film hydration.

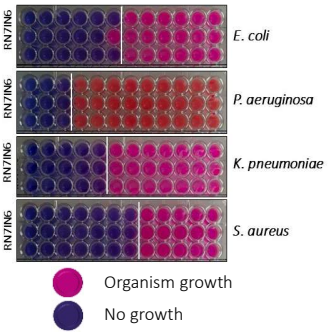


Fig. 4: Schematic representation of the MIC of RN7IN6.Vertical white lines indicate the MIC.

RN7IN6 was found to be effective against both Gram-positive and Gram-negative bacteria (Fig.4). MBC studies further showed that the AMP had a bactericidal action.

Conclusion

Synthesised RN7IN6 showed a promising, broad-spectrum bactericidal activity. Preliminary studies indicated that RN7IN6 could be surface adsorbed to bacteria-relevant liposomes, to produce a biomimetic delivery platform for a novel anti-infective agent. An increase in liposome size was noted following RN7IN6 incubation, while the surface charge was noted to decrease in magnitude, indicative of successful peptide adsorption.

Future work

- Optimisation of adsorption efficiency of RN7IN6 to biomimetic liposomes
- Investigation of RN7IN6 mechanism of action
- Production of AMP-loaded biomimetic liposomes via microfluidic mixing
- Assessment of antibacterial activity of RN7IN6-loaded biomimetic liposomes against a panel of wild-type and resistant bacteria.

References

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