Lipid-based Nanoformulations of Antimicrobial Peptides to Treat Bacterial Infectious Diseases

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Introduction

The rapid increase in drug-resistant infections presents an acute problem that continues to challenge the healthcare sector, generating interest in novel antimicrobial strategies. Antimicrobial peptides (AMPs) have a high potential as new therapeutics against infectious diseases as they are less prone to induce resistance due to their fast and non-specific mechanism of action. However the therapeutic potential of peptides is hampered by a number of chemical and biological concerns that impede their development. Hence the importance to protect the labile AMPs.

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Objective

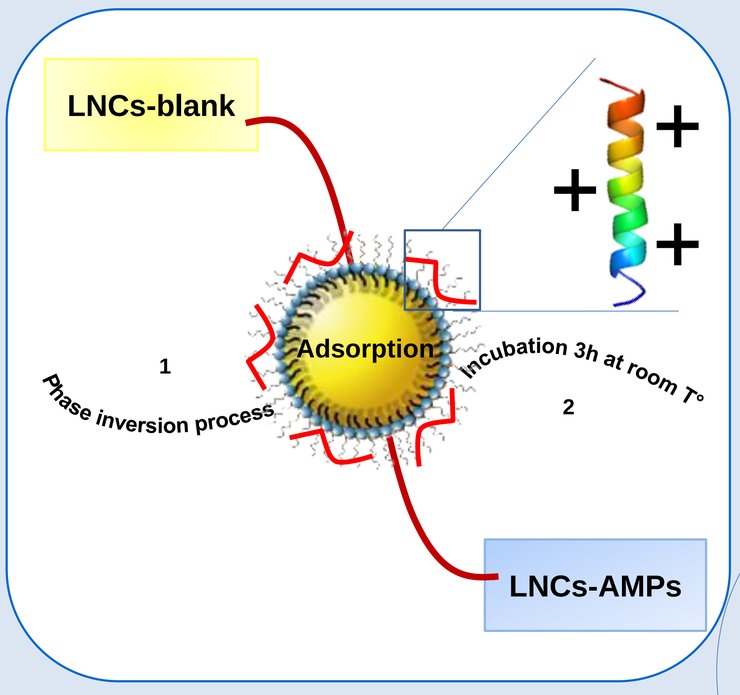
The aim of this study is to incorporate the peptide within the PEGylated shell of the Lipid Nanocapsules[1] (LNCs),this way the peptide can be nanopresented and protected against degradation.

The antimicrobial peptides

The peptides included in the study AMP1, AMP2 and AMP3 are well-defined AMPs, established to have an antimicrobial effect and an acceptable safety profile.

Experimental methods

Preparation of LNC-AMPs



Effect studies

The *in vitro* antimicrobial activity of the AMPs and nanoformulated AMPs was assessed against different Gram (-) and Gram (+) bacteria.

Which test?

- The determination of the minimal inhibitory concentrations[2] (MICs) via broth microdilution method.
- the time kill assay at a concentration corresponding to 2 times the MIC concentration of each formulation to investigate the kinetics of bacterial killing *in vitro.*

Bacterial strains

MIC? The lowest concentration of an antimicrobial agent that, under defined in vitro conditions, prevents the growth of a microorganism within a defined period of time

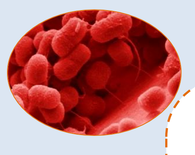
- Staphylococcus aureus (reference strain ATCC 25923)
- Methicillin-resistant Staphylococcus aureus MRSA (clinical strain 0702E0196)
- Pseudomonas aeruginosa (reference strain ATCC 27853)
- Pseudomonas aeruginosa (clinical strain 0704C0134)
- Escherichia coli (reference strain ATCC25922)
- ESBL Escherichia coli (clinical strain 9007550201)
- Acinetobacter baumannii (AYE ATCC BAA-1710)

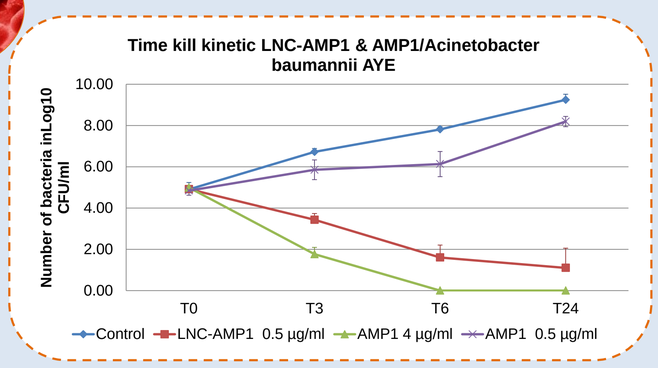
Results and discussion

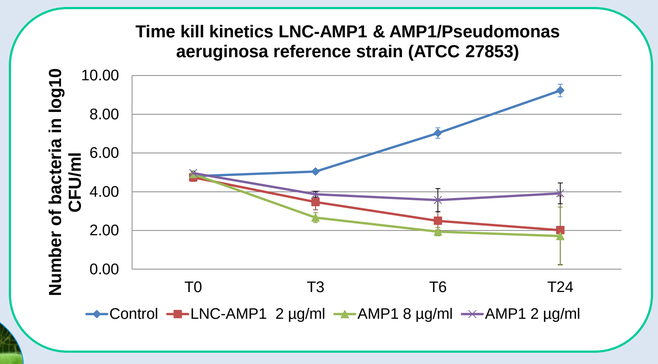
1. The activity of AMP1 against Gram- bacteria

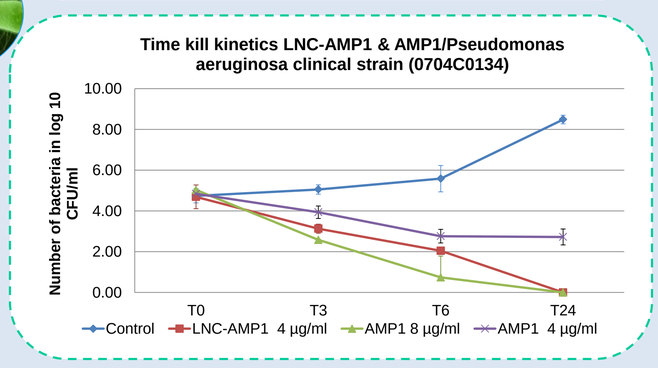
MIC (µg/ml)	AMP1	LNC-AMP1
P.aeruginosa reference strain	4	1*
P.aeruginosa clinical strain	4	2
E.coli reference strain	2	1
ESBL E.coli clinical strain	2	0.5*
A.baumannii AYE ATCC BAA-1710	2-4	0.25*

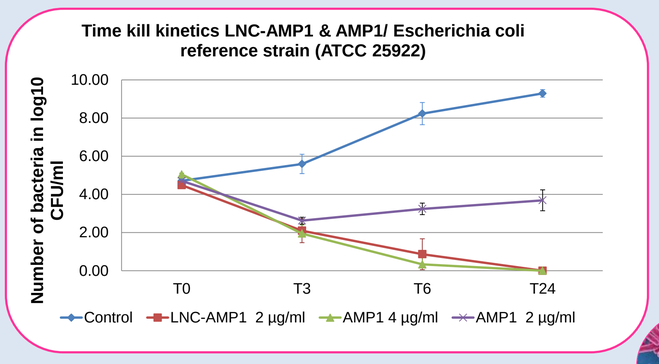
*Significant diffrence

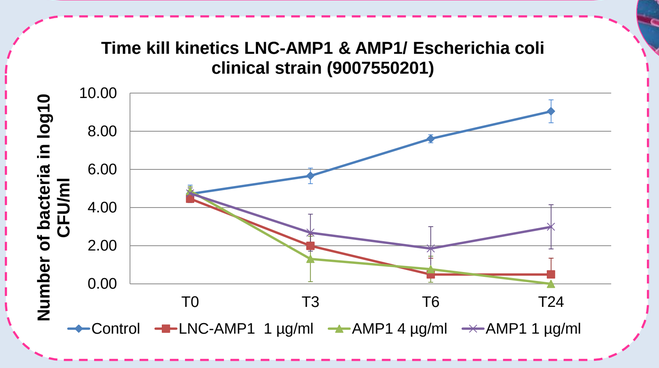








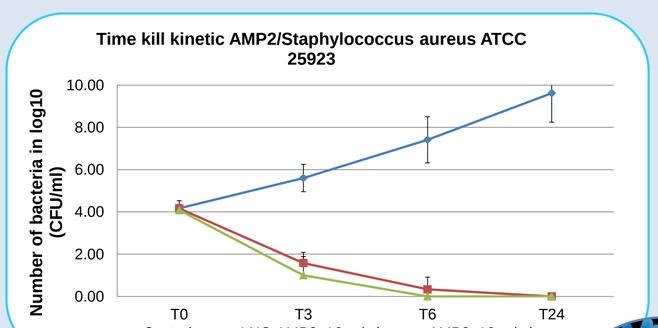


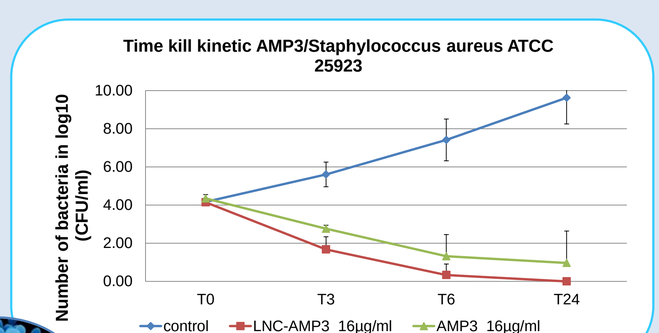


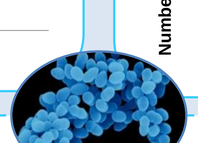
Results=mean(n=3) ± STD

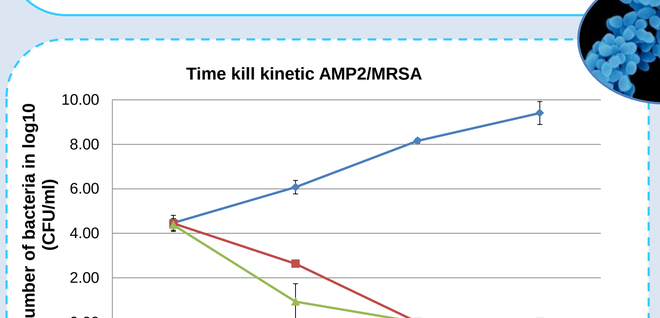
2. The activity of AMP2 and AMP3 against Gram+ bacteria

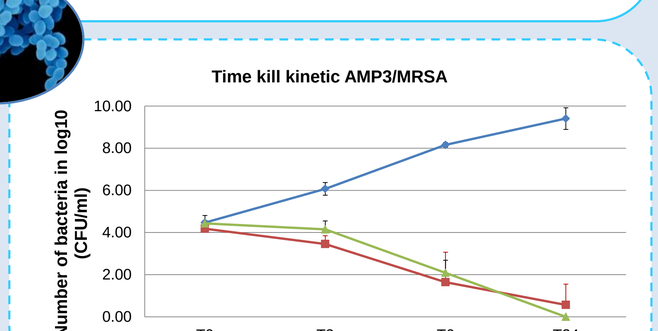
MIC (µg/ml)	AMP2	LNC-AMP2	AMP3	LNC-AMP3
S.aureus	8	8	8	8
MRSA	4	8	4	8











Results=mean(n=3) ± STD

Conclusion

The incubation performed with the different AMPs shows an efficient association of the peptide to the surface of the LNCs. The minimal inhibitory concentrations of the LNC-AMP were determined. In the case of AMP2 and AMP3, the results show a preservation of the anti-microbial activity of the native peptide against Staphylococcus aureus strains after association. The LNC-AMP1 show not only a preservation of the antimicrobial activity but more importantly, a significative lowering of the MIC with a similar bactericidal profil for the reference strain of Pseudomonas aeruginosa as well as the clinical strain of Eschirichia coli. These interesting results lead to more investigations to understand the nature of this potentiation. The stability of the nanoformulated AMPs will be assessed by protease assays.

References

1. Heurtault, B. et al. Pharmaceutical research 19, 875-880 (2002).
2. Andrews, J.M., et al. Journal of Antimicrobial Chemotherapy 48, 5-16 (2001).

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