Antibacterial activity of lipid nanocapsules and their interaction with antimicrobial peptides

Anita Umerska [1,2], Sergio Lociuro [3], Nada Matougui[1,2], Anne-Claire Groo [1,2], Viviane Cassissa[2], Patrick Saulnier[1], Marie-Laure Joly-Guillou[2]

1: INSERM U1066, University of Angers, France, 2: UPRESEA 3142, CHU Angers, France, 3: Adenium Biotech, Denmark.

Contact: anitaumerska@yahoo.fr

Introduction

Lipid nanocapsules (LNCs) are a new generation of biomimetic nanocarriers composed of an oily core of medium chain triglycerides that is surrounded by a shell composed of a lipophilic surfactant (lecithin) and a hydrophilic surfactant macrogol 15 hydroxystearate (1). The aim of the present study was to produce the LNCs with antibacterial activity by replacing lecithin with other lipophilic surface active compounds, namely medium chain fatty acids and their 1-monoglycerides which are known to possess antimicrobial properties (2,3,4). Another objective was to examine the interactions between the nanocapsules and the antimicrobial peptides (APMs), AP114 and AP138 (plectasin derivatives).

Experimental

Preparation of LNCs

LNCs were prepared via a phase inversion method at a concentration of 200 mg/ml. The LNCs were composed of a lipophilic surfactant (15%), macrogol 15 hydroxystearate (39%) and caprylic/capric acid triglycerides (46%).

Microorganisms

Staphylococcus aureus (reference strain ATCC 25923)(SA)

Methicillin-resistant *Staphylococcus aureus* (clinical strain 0702E0196) (MRSA)

P. aeruginosa, E.coli and *A. baumannii* (results not shown)

Minimum inhibitory concentration (MIC)

MIC was determined via a broth microdilution method in brain-heart infusion broth with the inoculum size of $1{,}65*10^6$cfu/ml.

Time-kill assay

The time-kill studies were performed with a final inoculum of approximately $3.3*10^4$cfu/ml in a final volume of 2 ml in a poly-propylene tube.

Results 1

Table 1. MIC of antimicrobial peptides AP138 and AP114.

	SA	MRSA
AP114	8 µg/ml	4 µg/ml
AP138	4 µg/ml	2 µg/ml

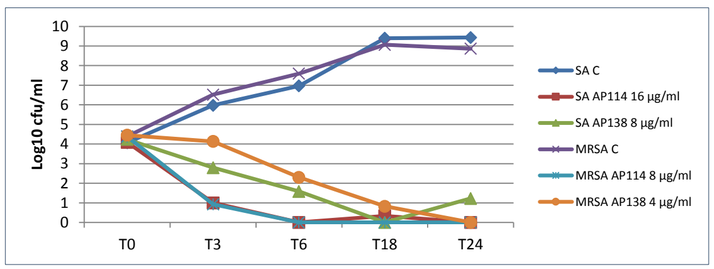

Figure 1 Time-kill curves of AP114 and AP138 against SA and MRSA.

Results 2

Table 2 MIC of blank lipid nanocapsules.

	SA	MRSA
Lecithin-LNCs	>50'000 µg/ml	>50'000 µg/ml
Caproic acid-LNCs	12'500 µg/ml	12'500 µg/ml
Caprylic acid-LNCs	6'250 µg/ml	6'250-12'500 µg/ml
Capric acid-LNCs	3'125 µg/ml	3'125-6'250 µg/ml
Lauric acid-LNCs	1'563 µg/ml	3'125 µg/ml
Monocaprin-LNCs	781-1'563 µg/ml	1'563 µg/ml
Monolaurin-LNCs	98-195 µg/ml	195-391 µg/ml

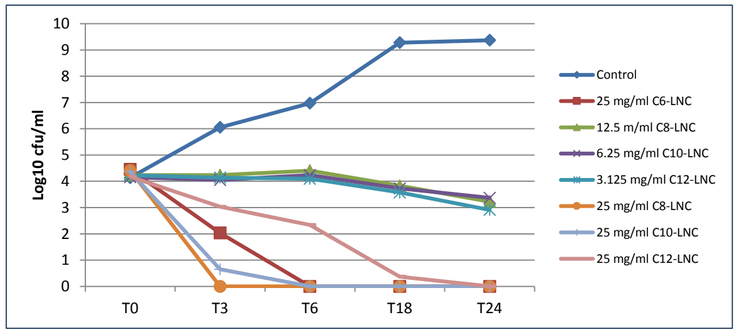

Figure 2 Time-kill curves of FA-LNCs against SA.

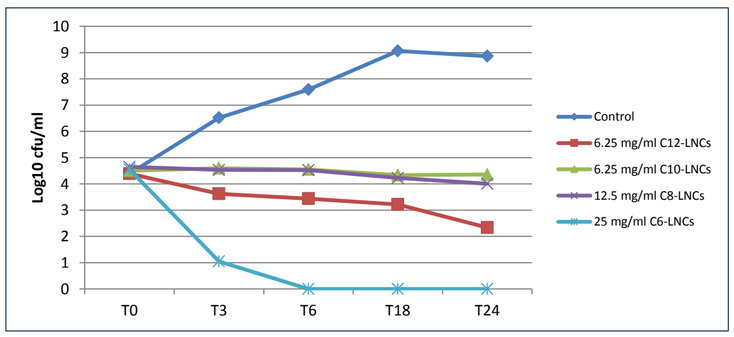

Figure 3 Time-kill curves of FA-LNCs against MRSA.

Results 3

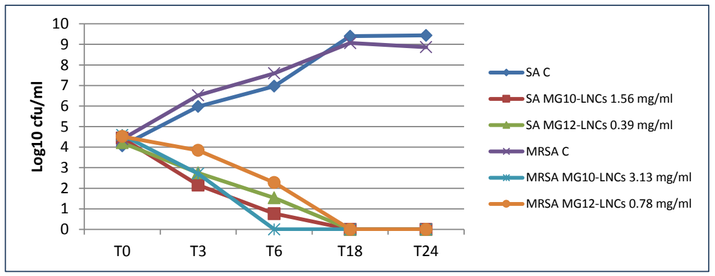

Figure 3 Time-kill curves of MG-LNCs against SA and MRSA.

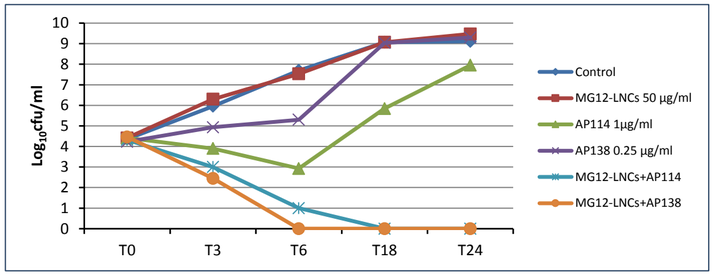

Figure 4. Time-kill curves of MG12-LNCs, AP114, AP138 and their combinations against MRSA.

Conclusions

- AP114 and AP138 were bactericidal against both *S. aureus* strains.
- Incorporation of medium chain fatty acids or their monoglycerides into the LNCs yielded nanovectors with antibacterial properties.
- *In vitro* synergy between monolaurin-LNCs and AMPs has been shown.

References

1. Huynh, N.T., Passirani, C., Saulnier, P., Benoit, J.-P., Int. J. Pharm. 2009; 379: 201-209
2. Batovska, D.I., Todorova, I.T., Tsvetkova, I.V., Nadjenski, H.M., Polish J .Microbiology 2009; 58: 43-47
3. Desbois, A.P., Smith, V.J., Appl. Microbiol. Biotechnol. 2010; 85:1629-1642
4. Huang, C.B., Alimova, Y., Myers, T.M., Ebersole, J.L., Arch. Oral Biol. 2011; 56:650-654

Acknowledgements

The study was funded by FORMAMP, a project within the EU Seventh Research Frame Programme (FP7) under grant agreement n°604182, in the area of nanotechnology. Antimicrobial peptides were provided by Adenium Biotech (Denmark).