THE DEVELOPMENT OF TOPICAL FORMULATIONS OF LIPOSOMAL MICROCOCCIN P1 FOR WOUND INFECTIONS

OSLO METROPOLITAN UNIVERSITY
STORBYUNIVERSITETET

B. F. Gurmessa[1], C. Charnock[1], K.V. Ovchinnikov[2], T. Thorstensen[2] & S. Nguyen[1]

[1] Department of Pharmacy, Faculty of Health Sciences, Oslo Metropolitan University, Oslo, Norway

https://doi.org/10.17952/38EPS.2026.P412

[2] Agribiotix AS, Ås, Norway

Contact information: sankon@oslomet.no

BACKGROUND

The antimicrobial peptide micrococcin P1 (MP1, fig. 1) has previously demonstrated antimicrobial activity against several clinically important gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), even on *S. aureus* biofilm, and showed no toxic effects against several human cell lines including skin keratinocytes[1-3]. As such, MP1 is a highly promising candidate for drug development, particularly targeting wound infections. However, MP1 is challenging to formulate due to its hydrophobic structure (fig.1) and poor water solubility. In this study, we develop topical formulations of MP1 entrapped in liposomes (fig. 2) and evaluated the antimicrobial effect of the formulations *in vitro*.

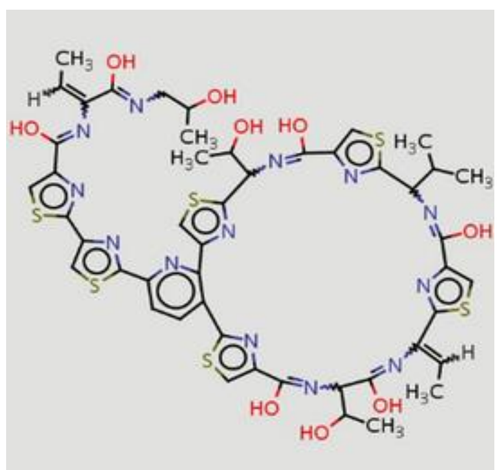


Fig. 1: The chemical structure of MP1.

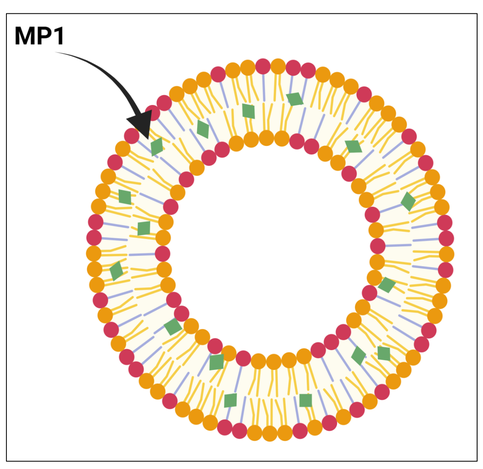


Fig. 2: MP1 (green squares) entrapped in the liposomal bilayer.

MATERIALS AND METHODS

MP1 (isolated from *S. equorum*, Agribiotix AS, Norway) was entrapped in conventional and fusogenic liposomes (phospholipids from Avanti Polar Lipids, AL, USA) by thin film-extrusion method and characterized by particle size, size distribution (polydispersity index, PDI), and zetapotential by dynamic light scattering (DLS), and MP1 entrapment efficiency (%EE) by high-performance liquid chromatography (HPLC). Unentrapped MP1 was separated by gel filtration (Sephadex G-25) before quantification and before incorporation into three semi-solid formulations; ointment, gel and cream, respectively. The ointment contained 60% vaseline, 20% liquid paraffin, 15% wool fat, and 5% cetyl alcohol (w/w, Apotekproduksjonen AS, Norway) and prepared by melting the solid/semi-solid components before adding liquid paraffin. The mixture was cooled down to room temperature under continuous stirring. The gel was prepared by gradually suspending 5% (w/w) hydroxypropylmethylcellulose (HPMC, Apotekproduksjonen AS, Norway) in water at room temperature under magnetic stirring (1000 rpm). The gel was left overnight to ensure complete swelling before use. Commercially available cream base was used (Apobase®, Karo Pharma, Sweden) for mixing with liposomes. The final topical formulations were prepared by adding 100 µl liposomal suspension to a gram semi-solid base and carefully mixed with an inoculating needle. The antimicrobial activity of the topical formulations was evaluated by disc diffusion method using *S. aureus* Xen29 (ATCC 112600, Revvity, MA, USA) as test organism. A commercially available antibiotic product containing fucidic acid (Fucidin® 2% ointment) was used as the positive control.

CONCLUSIONS

This study shows that liposomal entrapment and incorporation into semisolid systems did not impair MP1's antibacterial activity. However, the degree of activity is affected by the type of liposomes and semisolid base selected. For further pharmaceutical development of MP1, the most optimal formulation combination for the delivery to wound infections seems to be MP1 in fusogenic liposomes incorporated in gels.

RESULTS

Physicochemical characterization

Table 1 shows that both types of liposomes were positively charged and very similar in size and size distribution (both monodisperse), however, fusogenic liposomes could entrap more MP1 (35% corresponding to 0.175 mg/ml). Minimum inhibition concentration (MIC) of MP1 for *S. aureus* Xen29 has previously been shown to be 1-2 µg/ml[2] indicating 20-35% of MP1 entrapment in liposomes is sufficient to exert antibacterial activity. In addition, both types of liposomes demonstrated minimal changes in terms of particle size, size distribution and zeta potential when stored at 4° for 23 days (data not shown) indicating formation of stable nanoparticulate structures. Based on these results, both types of liposomes appears to be suitable nanocarrier systems for MP1 with fusogenic liposomes having higher entrapment of MP1.

Liposomal formulation	Particle size (nm in dm) ± S.D.	PDI	Zetapotential (mV) ± S.D.	% EE
MP1 in conventional liposomes (70% egg-PC/ 30% DOTAP)	90.7 ± 1.1	0.1	28.9 ± 0.3	20.0 ± 0.4
MP1 in fusogenic liposomes (50% DOTAP/25% DOPE/ 25% DAPC)	91.3 ± 0.6	0.1	35.4 ± 0.5	35.0 ± 0.4

Table 1: Physicochemical characterization of MP1-loaded liposomes. Egg-PC: L-α-phosphatidylcholine from egg (chicken), DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane, DOPE: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, DAPC: 1,2-diarachidoyl-sn-glycero-3-phosphocholine.

Evaluation of antimicrobial activity

The results from disc diffusion (Table 2) revealed that all formulations tested (MP1-loaded liposomes in semisolid formulations, MP1 alone in semisolid formulations, and MP1-loaded liposomes) showed measurable antibacterial activity compared to the negative control (no MP1 present) indicating that the topical formulations did not obstruct the MP1 release and activity. The zones of inhibition were higher for the gel-based formulations and significantly highest ($p < 0.05$) when MP1 was entrapped in fusogenic liposomes.

Topical formulation		Zones of inhibition (dm in mm) avg. ± S.D. n = 3
MP1-loaded conventional liposomes in:	ointment	9.33 ± 0.58
	cream	11.33 ± 0.58
	gel	12.33 ± 0.58
MP1-loaded fusogenic liposomes in:	ointment	10.67 ± 0.58
	cream	12.67 ± 0.58
	gel	**16.00 ± 1.00***
MP1 (without liposomes) in:	ointment	9.00 ± 1.00
	cream	11.00 ± 1.00
	gel	13.33 ± 0.58
MP1-loaded fusogenic liposomes	(alone)	15.67 ± 1.15
MP1-loaded conventional liposomes	(alone)	14.33 ± 0.58
Positive control (fucidic acid)	Fucidin® 2% ointment	27.0 ± 1.00
Negative controls (no MP1 present)	Ointment base	0
	Cream base	0
	Gel base	0

Table 2: Zones of inhibition for different formulations with and without MP1 against the test organism *S. aureus* Xen29 determined by disc diffusion. * indicate statistically significant difference between MP1-loaded conventional liposomes and MP1 without liposomes within the same type of semisolid formulation (one-way ANOVA, $p > 0.05$).

References

1. K. V. Ovchinnikov et al., Antimicrob. Agents Chemother. 2020, 64, 10-1128.
2. A. M. Amer et a., Eur. J. Pharm. Sci. 2025, 211, 107149.
3. Y. Liu et al., Microbiome 2020, 8, 85.

Acknowledgements

This work was supported under grant # 341669 by Research Council of Norway