Influence of Oligo-Peptides on Fluconazole – loaded DPPC Liposomes: Improvement of Size and Stability

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Introduction

Liposomes are colloidal delivery systems which are frequently used in pharmaceutical formulations or cosmetic products. Their ability to deliver both hydrophilic and lipophilic substances into and across the skin makes them interesting vehicles for various applications.
However, their physicochemical long-term stability is rather poor. Therefore, the present study focuses on the improvement of liposomal properties and skin interaction by the incorporation of oligo-peptides such as Lys-5. This novel approach might open new perspectives in terms of improved liposome development.

Experimental Methods

Development of the formulations
Liposomes were created by the thin-film hydration method, a standard procedure for liposome preparation.The oligo-peptides were developed using the solid phase peptide synthesis technique.

Stability assessment
The physicochemical stability of the formulations was investigated in terms of particle size and zeta potential. The mean particle size as well as the polydispersity index were measured by photon correlation spectroscopy. The zeta potential was determined by laser Doppler electrophoresis.

Skin permeation
The skin permeation of the formulations was examined using Franz-typed diffusion cells with phosphate buffer pH 6,8 as acceptor medium. Porcine skin was used as a model membrane. Samples were drawn after 2, 4, 6, 8 and 24 hours. The permeated amount of fluconazole was determined by HPLC.

Results

The particle sizes of the formulations were between 30 - 80 nm. The incorporation of an oligo-lysine always resulted in an decrease in size compared to the unloaded liposomes. Even smaller liposomes could be prepared when fluconazol was added to the oligo-lysine-loaded liposomes. Moreover, also the polydispersity index of these small liposomes satisfied our expectations with values around 0,05.
For the sake of completeness the zeta potential of the formulations was evaluated also and the values ranged from 6 to 20 mV.
The stability over 6 weeks of selected formulations was determined. The formulations with 5% Lys-5 and fluconazole showed the best mean particle size and polydispersity index. Thus, its stability was compared to the corresponding formulations without oligo-peptide. The formulation with 5% Lys-5 and fluconazole exhibited constant small particle sizes and PDI, while the liposomes without Lys-5 grew larger and more polydisperse with storage time.
Skin permeation experiments were performed and all formulations with oligo-peptides showed satisfying skin permeation rates in vitro.

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Formulation	MPS [nm]	PDI	pH
7.5% DPPC	61.49 ± 2.74	0.294 ± 0.001	3.6
3.75% DPPC	72.43 ± 12.48	0.249 ± 0.029	4.0
3.75% DPPC 0.5% Fluc	67.28 ± 15.86	0.238 ± 0.095	4.1
3.75% DPPC 5% Lys-5	45.83 ± 10.08	0.142 ± 0.075	3.0
3.75% DPPC 5% Lys-5 0.5% Fluc	37.00 ± 1.80	0.138 ± 0.053	3.2
3.75% DPPC 10% Lys-5	56.51 ± 11.95	0.155 ± 0.079	2.7
3.75% DPPC 10% Lys-5 0.5% Fluc	44.77 ± 2.91	0.115 ± 0.046	2.9
3.75% DPPC 5% Lys-7	51.93 ± 5.12	0.172 ± 0.055	2.8
3.75% DPPC 5% Lys-7 0.5% Fluc	45.09 ± 4.60	0.123 ± 0.038	2.9
3.75% DPPC 10% Lys-7	66.95 ± 10.99	0.219 ± 0.027	2.6
3.75% DPPC 10% Lys-7 0.5% Fluc	61.15 ± 4.25	0.186 ± 0.042	2.8

Table 1: Liposome properties. Influence of oligo-peptides on mean particle size (MPS), polydispersity index (PDI) and pH.

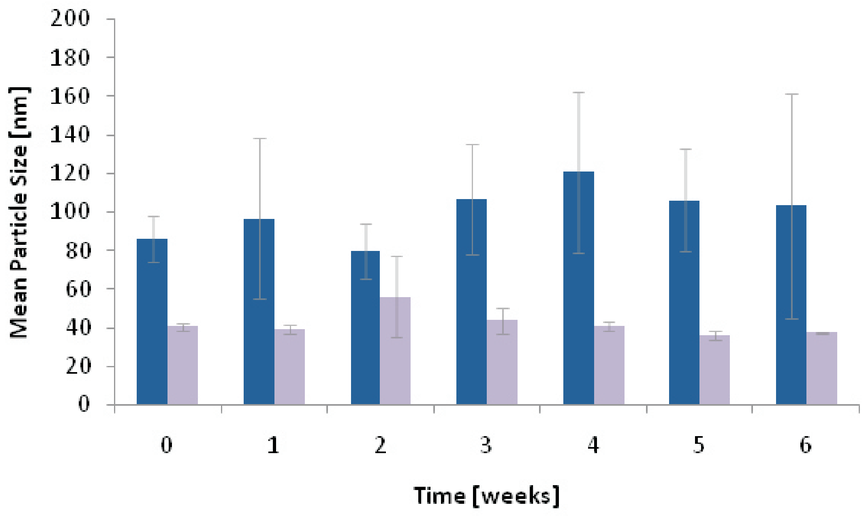

Figure 1: Particle size of liposomes with fluconazole. Blue bars: without oligo-peptide, purple bars: with 5% Lys-5.

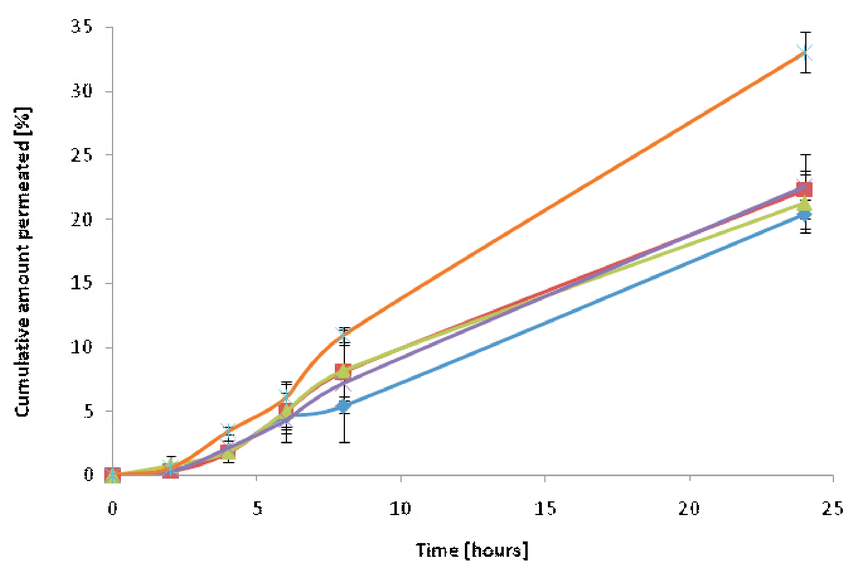

Figure 2: Skin permeation rates of fluconazole from liposomes with and without oligo-peptides. Orange line: 0.5% Fluconazole, Blue line: 5% Lys-5 + 0.5% Fluconazole, Red line: 10% Lys-5 + 0.5% Fluconazole, Green line: 5% Lys-7 + 0.5% Fluconazole, Purple line: 10% Lys-7 + 0.5% Fluconazole

Conclusion

Liposomal formulations were improved by the incorporation of oligo-peptides.The mean particle size as well as the polydispersity index of the liposomes were reduced. This parameters also remained constant over a storage period of six weeks. In contrast, the liposomes without oligo-peptides showed larger particle sizes and higher polydispersity indeces. Further work should involve the tape stripping method in order to evaluate the penetration properties of the formulations with oligo-peptides into the skin.