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Friedrich-Schiller-Universität Jena
Department of Pharmceutical Technology

Penetration studies with temoporfin-loaded liposomes: invasomes versus conventional liposomes

Nina Dragicevic-Curic[1,2], Dietrich Scheglmann[3], Volker Albrecht[3], Alfred Fahr[1]
[1]Department of Pharmaceutical Technology, Friedrich Schiller University Jena, Lessingstrasse 8, 07743 Jena
[2]Apoteka „Beograd", Bojanska 16/IV, 11000 Belgrade, Serbia
[3]Biolitec AG, Otto-Schott-Strasse 15, 07745 Jena

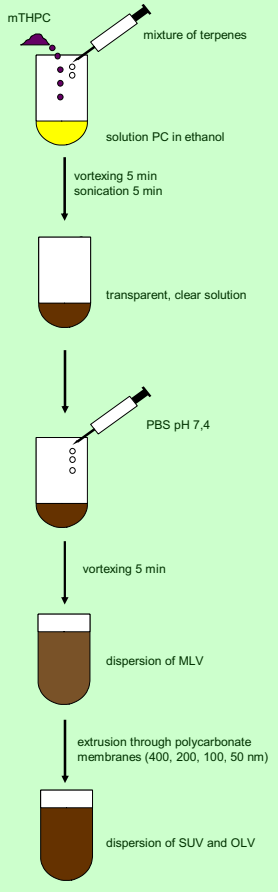
Introduction

Invasomes represent a novel liposomal carrier system for enhanced skin delivery. They contain besides phosphatydilcholine and the aqueous phase, also small amounts of ethanol and terpenes or terpene mixtures.
Since temoporfin (mTHPC) is a highly hydrophobic second generation photosensitizer with low percutaneous penetration, the aim of this study was to develop a carrier system able to enhance the percutaneous penetration of mTHPC.

Materials

The soybean phosphatidylcholine solution in ethanol (NAT 8539) was a gift from Phospholipid GmbH (Cologne, Germany). Terpenes d-limonene, citral and 1,8 cineole were purchased from Sigma-Aldrich (Germany). Temoporfin, i.e. 7,8-dihidro-5,10,15,20-tetrakis-(3-hydroxyphe-nyl) porphyrin (mTHPC) was a gift from Biolitec AG (Jena, Germany). All other chemicals were of analytical grade. The LiposoFast® mini-extruder used for the extrusion was from Avestin (Ottawa, Canada).

Methods



Scheme 1. Schematic representation of the preparation of invasomes with and without PE.

Preparation

mTHPC-loaded invasomes were prepared (Scheme 1) by dissolving 1.5 mg/ml of the hydrophobic drug mTHPC and 0-1% w/v of the mixture of terpenes [cineole:citral:d-limonene = 45:45:10 v/v (PE = penetration enhancer)] in the ethanolic phospholipid solution (SPC:ethanol = 75:25 w/w). The mixture was vortexed for 5 min and afterwards sonicated for 5 min. PBS was added to the solution by a syringe under constant vortexing. The last step was the extrusion of multilamellar vesicles (MLV) through polycarbonate membranes of different pore sizes (400 nm, 200 nm, 100 nm, 50 nm).

Characterization

The particle size, polydispersity index (PDI) and zeta potential of the invasomes were determined by dynamic light scattering using the Zetasizer NS (Malvern Instrument, Herrenberg, Germany).

Cryo-electron microscopy

The obtained invasomes were visualized by cryo-electron microscopy. Five microliters of invasome dispersions were put onto a perforated coated net of copper (Quantifoil R 1.2/1.3, 400 mesh). The excess of samples was removed with a sheet of filter paper. Samples were quickly frozen with liquid ethane (-170 to -180 °C) in a cryo-box (Carl Zeiss NTS GmbH). The excess of ethane was removed and the samples were placed by a cryo-transfer device (Carl Zeiss NTS GmbH) in the precooled transmission-cryo-electron-microscope (Philips CM 120). Microscopy was performed at 120 kV.

Penetration studies

The penetration studies were performed *in vitro* using human full-thickness abdominal skin. Finite-doses of formulations (10 µl/cm2) were applied non-occlusively onto the skin for 6 h. After 6 h a sample of 1 ml was withdrawn from the acceptor solution and analyzed for the mTHPC-content. Formulations were then removed from the skin and tape-stripping of the stratum corneum and cryosectioning of the deeper skin layers were performed in order to determine the mTHPC-amount penetrated into different skin layers. The mTHPC-content in the samples was analyzed by HPLC.

Results and discussion

Particle size and zeta potential

The particle size of invasomes was in the range of 82.7 ± 0.3 to 124.3 ± 0.6 nm, and it increased with increasing the amount of PE in the vesicles (Fig. 1). The PDI (Fig. 1) of the investigated vesicles had values from 0.086 ± 0.004 (high homogeneity) to 0.227 ± 0.003 (lower, but acceptable homogeneity, since PDI < 0.3). Regarding the zeta potential, invasome dispersions possessed a negative surface charge and with increasing the amount of PE in the vesicles the negative surface charge increased slightly (Fig. 2).

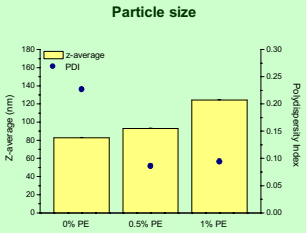


Fig. 1. Particle size and PDI of invasomes.

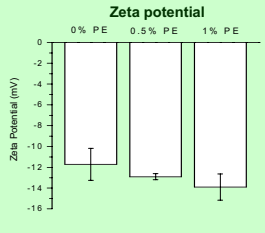


Fig. 2. Zeta potential of invasomes.

Cryo-electron microscopy

Electron microscopy revealed that the vesicles were mostly unilamellar (Fig. 3 a,c,e, black short arrows) and bilamellar (Fig. 3 a,c,e, black medium length arrows), spherical and oval in shape, but also oligolamellar vesicles were observed (Fig. 3 b,d, black long arrows). In invasome dispersions with 0.5% and 1% PE also deformed vesicles were present (Fig. 3 c,f, red arrows).

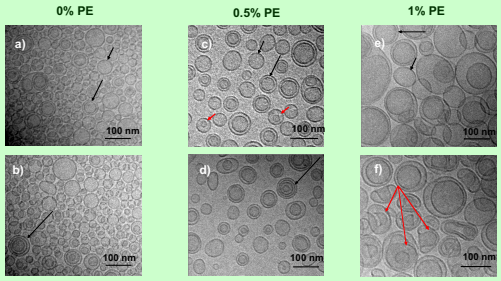


Fig. 3. Visualization of invasomes with 0-1% PE by cryo-electron microscopy. (a, b) invasomes with 0% PE,, (c, d) invasomes with 0.5% PE, (e, f) invasomes with 1% PE. Black short arrows represent unilamellar vesicles, black arrows of medium length represent bilamellar vesicles, while black long arrows represent oligolamellar vesicles. Red arrows represent deformed vesicles.

Penetration studies

Invasomes with 1% terpenes (i.e. 1% PE) revealed a significantly enhanced accumulation of mTHPC in the SC compared to liposomes with 3.3% ethanol (i.e. 0% PE) and invasomes containing 0.5% terpenes (i.e. 0.5% PE), showing a direct relationship between the amount of terpenes in vesicles and the penetrated mTHPC-amount (Fig. 4, Table 1). In comparison to conventional liposomes (with 0% ethanol), invasomes with 1% terpenes delivered a 3.87-fold higher mTHPC-amount to the SC. These invasomes delivered also a sufficiently high mTHPC-amount to the deeper skin layers.

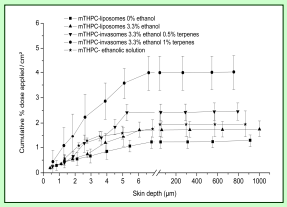


Fig. 4. Skin depth profile of mTHPC from different mTHPC-liposomes.

Table 1. Amounts of mTHPC delivered from liposomes and invasomes to different layers of human skin (n = 3).

Formulation	SC		Deeper skin		SC+deeper skin*		SC	Deeper skin
	% dose/cm²	ng/cm²	% dose/cm²	ng/cm²	% dose/cm²	ng/cm²	ng/mg tissue	ng/mg tissue
Liposomes 0% ethanol	1.03±0.14	160±13	0.045±0.019	7.05±2.35	1.07±0.14	167±11	233±41	0.097±0.036
Liposomes 3.3% ethanol	1.72±0.32	207±22	0.031±0.017	4.34±2.46	1.76±0.33	212±21	344±37	0.073±0.043
Invasomes 3.3% ethanol 0.5%PE	2.42±0.32	335±39	0.040±0.024	5.59±3.53	2.46±0.34	341±42	644±106	0.089±0.050
Invasomes 3.3% ethanol 1.0%PE	3.99±0.70	561±127	0.038±0.018	5.22±2.19	4.02±0.69	566±125	856±174	0.095±0.022
Ethanolic solution	1.95±0.53	276±91	0.003±0.002	0.43±0.34	1.95±0.53	277±91	430±168	0.008±0.006

*deeper skin = viable epidermis and dermis.

Conclusion

Vesicles in invasome dispersions were of adequate particle size and homogeneity, mostly unilamellar and bilamellar, oval and spherical in shape. Also a high number of deformed vesicles was seen in the invasome dispersion with 1% terpenes (i.e. 1% PE), which should be further investigated. Invasomes with 1% terpenes showed the highest penetration enhancing ability.

Acknowledgements

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