PENETRATION MECHANISM OF CLASSICAL AND DEFORMABLE LIPOSOMES THROUGH PIG EAR SKIN

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PURPOSE

Deformable liposomes have been developed and evaluated as novel topical and transdermal delivery system. Their mechanism of drug transport into and through the skin has been investigated but remains an area of contension[1]. This present work concerns *ex vivo* diffusion studies using pig ear skin in order to explain the penetration mechanism of classical and deformable liposomes.

METHODS AND RESULTS

Liposomes preparation and characterisation

Classical liposomes were made of phosphatidylcholine (PC). Deformable liposomes contained in addition an "edge activator" at 13 % m/m concentration. Charged liposomes contained stearylamine (SA) or dimyristoyl phosphatidic acid (DMPA). Liposomes were prepared by the classical film evaporation method. Two types of vesicles were studied; the first one encapsulated betamethasone (BMS) in the aqueous compartment by the use of BMS-cyclodextrin inclusion complexes (PC-HPγCD-BMS) while the second one encapsulated BMS in their lipid bilayer (PC-BMS). Liposome stability was evaluated by measuring the leakage of encapsulated BMS (Fig. 1).

*Table 1: Size, deformability index, Zeta potential and encapsulation efficiencies ± S.D. (n = 3, *n=1, ND=not determined).*

Composition	Size (nm)	Deformability index	Zeta Pot (mV)	$EE_{BMS/lip}$(%)	$EE_{BMS/BMStot}$(%)
PC -HPγCD-BMS	222 ± 5	3.4 ± 0.3	-2.0 ± 1.9*	1.57 ± 0.07	39.1 ± 1.9
PC-BMS	222 ± 10	5.2 ± 1.0	ND	3.96 ± 0.27	97.8 ± 5.4
PC-Na deoxy-HPγCD-BMS	191 ± 7	8.2 ± 0.6	-7.8 ± 1.9*	1.98 ± 0.09	42.5 ± 2.0
PC-Na deoxy-BMS	193 ± 23	8.0 ± 0.4	ND	3.43 ± 0.15	74.6 ± 2.5
PC-Tween 80®-BMS	167 ± 10	7.5 ± 0.2	ND	4.36 ± 0.06	92.0 ± 3.4
PC-Span 80®-BMS	171 ± 10	2.8 ± 0.2	ND	4.24 ± 0.32	89.5 ± 6.5
PC-DMPA -HPγCD-BMS	207 ± 20	ND	-31.5 ± 6.1	2.04 ± 0.15	44.3 ± 3.2
PC-SA-HPγCD-BMS	160 ± 12	ND	+17.6 ± 1.4	1.87 ± 0.10	44.7 ± 2.4
PC-DMPA-BMS	205 ± 11	2.3 ± 0.1	-26.6 ± 3.5	4.52 ± 0.08	96.9 ± 1.7
PC-SA-BMS	140 ± 11	2.0 ± 0.2	+13.3 ± 2.2	3.97 ± 0.03	92.2 ± 1.2

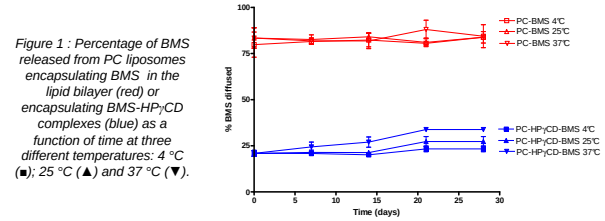

Figure 1 : Percentage of BMS released from PC liposomes encapsulating BMS in the lipid bilayer (red) or encapsulating BMS-HPγCD complexes (blue) as a function of time at three different temperatures: 4 °C (■); 25 °C (▲) and 37 °C (▼).

The encapsulation of BMS by the use of cyclodextrins enhanced the stability of the formulation. The percentage of BMS diffused from classical liposomes was reduced from 83.8% to 23.3% by the use of cyclodextrins after one month at 4°C (Fig. 1).

Ex vivo diffusion studies

Ex vivo studies were carried out using pig ear skin because of its similarity to human skin in terms of morphology and permeability characteristics, making it a practical alternative to human skin. Tape stripping method was used to determine BMS diffusion in the stratum corneum. Solid phase extraction was needed to clean up sample from diffusion studies before liquid chromatography analysis. This analytical method was successfully validated, based on an accuracy profile approach[2].

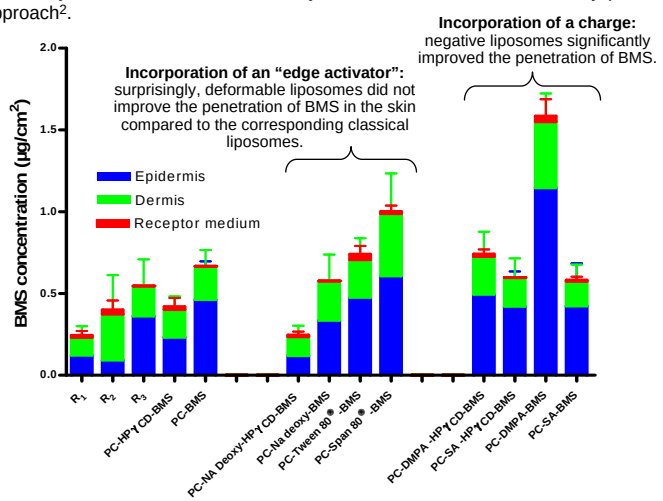

Figure 2: Concentration of BMS diffused (µg/cm²) (± S.D.) in the epidermis, dermis and receptor medium of Franz's cells after 24h from 350 µL of formulation containing BMS (150 µg/mL) through pig skin , in non occlusive condition (n=9). R_1: BMS in ethanol solution, R_2: BMS-HPγCD solution, R_3: dispersion of PC and BMS in Hepes buffer.

The encapsulation of BMS-cyclodextrin complexes reduced the percentage of BMS in the epidermis from 0.46 µg/cm² to 0.23 µg/cm² ($p<0.05$) (Fig.2). The reservoir effect of liposomes containing cyclodextrins contributes to the reduced penetration.

The high permeability of liposomes containing BMS in their lipid bilayer and the better penetration of this formulation argued against the passage of BMS through the stratum corneum under the encapsulated form.

There was no significant difference in diffusion between the dispersion of PC and BMS (R_3) and the PC-BMS liposomes. On the other hand, R_3 diffused better in the epidermis than R_1 and R_2 ($p<0.001$) (Fig. 2), indicating that PC acts as a penetration enhancer.

Confocal microscopy

Liposomes were made fluorescent by two ways:

- Aqueous cavity by inclusion of calcein
- Lipid bilayer by incorporation of NBD-phosphatidylcholine (NBD-PC)

Confocal microscopy results are in agreement with those of the diffusion studies and can highlight a penetration of the liposomes into the hair follicles. The confocal microscopy study shows that liposomes do not penetrate intact into the skin. Calcein remains in the upper layers of the skin, in the stratum corneum while NBD-PC penetrates deeply in the epidermis and the dermis.

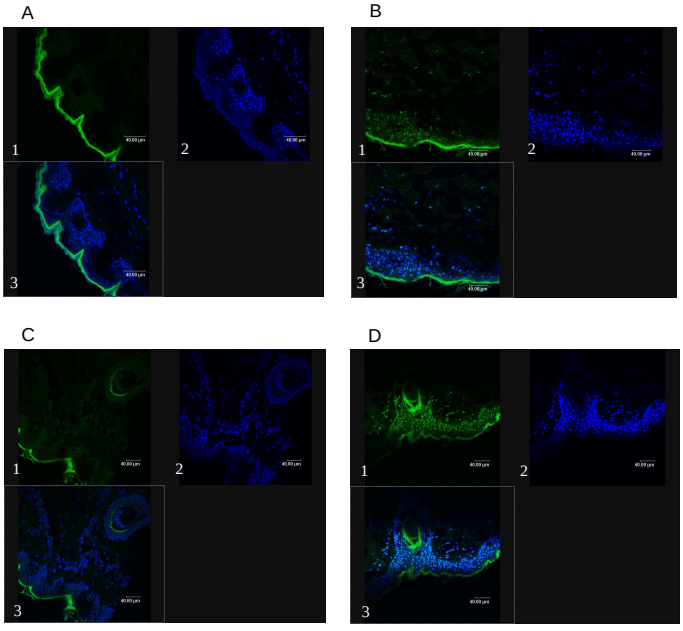

Figure 3 A, B, C and D: CLSM images, each image divided in three parts with 1: fluorescence due to calcein or NBD-PC, 2: fluorescence of keratinocytes nuclei; 3: superposition of 1 and 2. A and C: PC liposomes encapsulating calcein; B and D: PC liposomes encapsulating NBD-PC. Bar represents 40 µm.

CONCLUSIONS

Results showed that liposomes do not penetrate intact into the deeper layers of the skin. PC acts as a penetration enhancer. BMS molecules are released from the vesicles after which free drug molecules can diffuse through the stratum corneum and partition in the viable skin tissue. In this study, deformable liposomes do not improved the penetration of BMS compared to classical liposomes. The Zeta Potential of the vesicles seems to play an important role.

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