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Enhanced Skin Permeability of Lipophilic and Hydrophilic Drugs via Rigid-, Elastic- and Conventional Liposomes: A Comparative Study

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Introduction

Several researchs reported the role of lipid compositions on liposomes for transdermal delivery. Since the report about the success of transfersomes to deliver model drug into deep skin region was published,[1] the novel high elastic vesicles (such as ethosomes,[2] flexosomes,[3] invasomes,[4, 5] menthosomes[6] and transinvasomes[7]) that can squeeze themselves through the outermost layer of the epidermis (stratum corneum) which much smaller pore than their vesicle size, have been designed and developed. Transfersomes, a prime generation of elastic liposomes (ELP) for transdermal delivery of various drugs was also developed and introduced by Cevc et al. The major mechanism of ELP to enhance the skin permeation was presented by edge activator and/or single-chain surfactant single-chain surfactant. Several researchs suggested that elastic vesicles were more efficient in enhancing the transport of drugs than conventional vesicles. The physical stability of the new generation of elastic vesicles may be poor as the fluidity of its bilayer. The opportunities in transdermal delivery of elastic liposomes are still challenging. To improve the stability of conventional liposomes (CLP), the incorporation of cholesterol as membrane stabilizer was introduced. The previous study reported the addition of cholesterol to rigid liposomes (RLP) successfully improved the membrane stability.[8] However, the proper ratio between edge activator and membrane stabilizer was not fully determined. An increase in edge activators can lead to the increase of the skin permeability but the stability of bilayer may decrease as its fluidity. On the other hand, the cholesterol not only increased the stability and rigidity, but also simultaneously decreased the permeability of the lipid bilayer.[9] In research and development of pharmaceutical products, the major priority consideration was the safety, the efficacy and the stability of the formulation. Thus, the objectives of this study were to investigate the influence of cholesterol and dicetyl phosphate on the physicochemical characteristics of different vesicle formulations, and to compare the skin permeability of RLP, ELP and CLP for meloxicam (MX) (lipophilic drug) and sodium fluorescein (NaF) (hydrophilic drug). The physicochemical characteristics, e.g. vesicle size, surface charge, entrapment efficiency, and skin permeability of the MX and the NaF loaded vesicle formulations were evaluated and compared.

Methods

Liposomal preparation

The lipid vesicle formulations of RLP, ELP and CLP composed of a constant amount of MX and/or NaF, 10 mM phosphatidylcholine (PC) and various amount of 1 mM cholesterol (Chol) (as the membrane stabilizer) and various amount of dicetyl phosphate (DCP) (as the penetration enhancer) were prepared by thin film hydration method (Table 1). MX was added in lipid phase before the preparation of the lipid thin film. The dried lipid film containing MX was hydrated with phosphate buffer solution (PBS; pH 7.4), while NaF in phosphate buffer solution was added in blank-dried lipid film for preparing NaF loaded vesicles. All vesicle formulations were subsequently sonicated for two cycles of 15 min using a probe-type sonicator (5510J-DTH Branson Ultrasonics, Danbury, U.S.A.). The MX and NaF loaded vesicle formulations were freshly prepared and stored in airtight containers at 4°C prior to use.

Vesicle size, size distribution and zata potential determination

Average vesicle size and surface charge of the vesicles were measured by photon correlation spectroscopy (PCS) (Zetasizer Nano series, Malvern Instruments, U.K.). All measurements were investigated at room temperature (25 °C), after diluting the vesicle formulations. Twenty microlitres the sample formulations were diluted with 1480 µL of deionized water. At least three independent samples were taken, and thevesicle size, size distribution and zeta potential were measured at least three times.

Entrapment efficiency determination of MX

The concentration of MX in the formulation was determined by HPLC analysis after disruption of the vesicles with Triton® X-100 (0.1% w/v) at a 1:1 volume ratio and appropriate dilution with phosphate buffer solution (pH 7.4). The vesicles/Triton® X-100 solution was centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was filtered through a 0.45 µm nylon syringe filter. The entrapment efficiencies (EE) of MX loaded vesicle formulations were calculated using this equation (1):

$$EE = 100 \times \frac{The\ concentration\ of\ drug\ in\ vesicles}{The\ initial\ concentration\ of\ drug\ added} \quad (1)$$

Entrapment efficiency determination of NaF

The 0.5 mL of vesicle formulation was placed in an ultrafiltration tube with a molecular weight cutoff of 3000 Da *(*Microcon YM-3; Minipore, Billerica, MA, U.S.A.*)* and centrifuged at 4°C at 10000×g for 60 min. The filtrate was discarded, then 0.25 mL of phosphate buffer solution was added before further centrifugation at 4°C at 10000×g for 40 min. The collected NaF loaded liposomes in the retentate were then disrupted with 0.2 mL of Triton® X-100 solution and final centrifuged at 4°C at 10000×g for 10 min. The NaF concentratoin was determined by fluorescence analysis and calculated using equation (1).

In vitro skin permeation study

The shed snake skin of Siamese cobra (*Naja kaouthia*) was used as a model membrane for the skin permeation study because of its similarity to human skin in lipid content and permeability[10]. A side by side diffusion cell with an available diffusion area of 0.95 cm^2 was operated. The donor and receiver chamber was filled with 3 mL of tested formulation and 3 mL of phosphate buffer solution (pH 7.4, 37°C), respectively. At time intervals; 2, 4, 6 and 8 h, 1 mL of the receiver fluid was withdrawn, and the same volume of fresh buffer solution was placed in the receiver chamber. The concentration of MX and NaF was determined using HPLC and fluorescence analysis, respectively.

Data analysis

The data are reported as the means ± standard deviation (SD) (n=3). A *p*-value of less than 0.05 was considered to be significant.

Table 1. The composition of different liposome formulations

Formulation	Lipid composition (%W/V)					PBS pH 7.4 qs to
	PC	Chol	DCP	MX	NaF	
RLP	0.77	0.04	-	0.07	0.21	100 mL
ELP	0.77	0.04	0.10	0.07	0.21	100 mL
CLP	0.77	-	-	0.07	0.21	100 mL

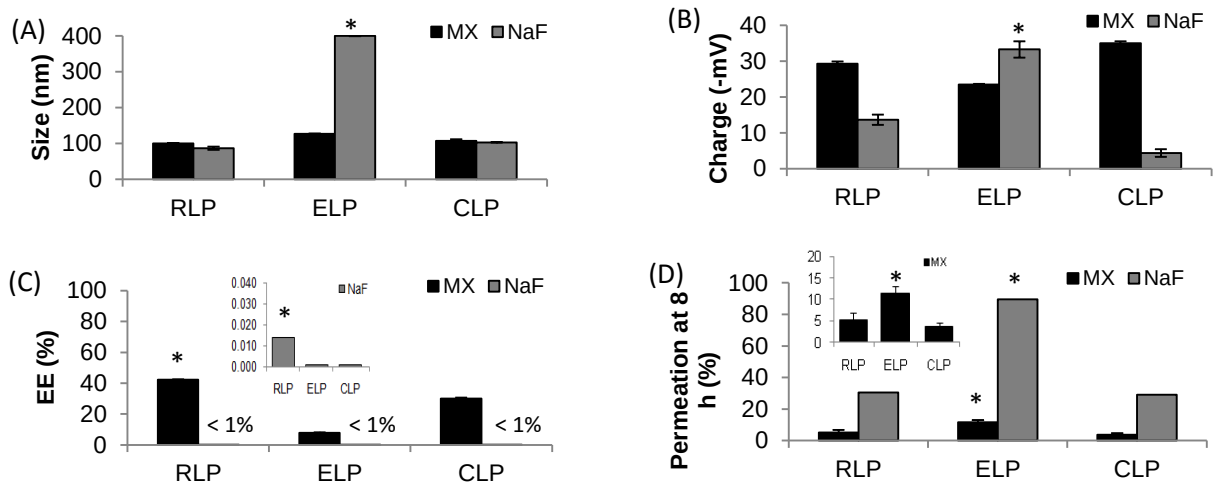

Figure 1. The physicochemical characteristics of vesicle formulations; (A) vesicle size, (B) surface charge, (C) entrapment efficiency (EE) and (D) permeation at 8 h.

Results and Discussion

Physicochemical characteristics of vesicle formulations

The vesicle size, surface charge, entrapment and permeation of vesicle formulations were shown in Figure 1. The addition of cholesterol cause the significant differencs in physicochemical characteristics e. g. entrapment efficiency and permeation of MX and NaF loaded vesicle formulations (RLP, ELP, CLP). The vesicle size of all MX loaded vesicle formulation was smaller than 200 nm with a narrow size dispersion, while the vesicle size of NaF- ELP was significantly larger than other NaF loaded vesicle formulations. Although other previous study[11] indicated the influence of lipid composition on the vesicle size, but in this study the vesicle size might also be influenced by the method of preparation. However, the vesicle size of most liposome formulations was in nanosize range with the narrow distribution. Regarding to the anionic species of MX, NaF and DCP, the surface charge of all loaded vesicles was negative, depending on the intrinsic properties of vesicle compositions and total net charge. The isoelectric point (PI) of the phosphatydylcholine (PI = 6) was lower than the experimental pH 7. 4, thus it should be presented as the cationic species in this study. However, the total net charge of all vesicle compositions was negatively charged.

The entrapment efficiency of MX loaded RLP was significantly higher than CLP and ELP, respectively. This result may be attributed to the increase of cholesterol (as the lipophilic portion) in the bilayer of vesicles. Moreover, the previous study reported that the drug content increased when surfactant was added.[12] Nevertheless, the addition of DCP resulted in the decrease of entrapment efficiency of MX. Considering the intrinsic properties of DCP, the lipophilic moiety was inordinate as its large molecular structure. The competition between lipophilic moiety of MX and DCP might occur in the vesicle bilayers. Thus MX loaded ELP showed a low entrapment efficiency. However, the entrapment efficiency of MX loaded vesicles was significantly higher than NaF loaded vesicle formulations (less than 1%).

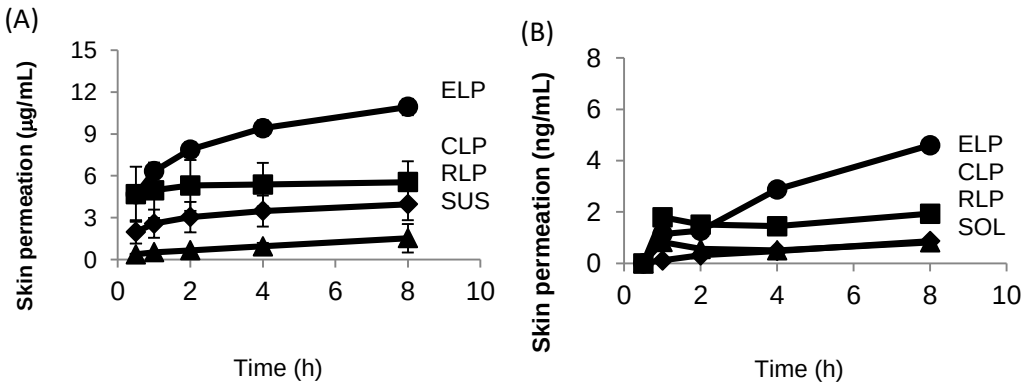

Figure 2. The skin permeation profile of (A) MX loaded vesicles and (B) NaF loaded vesicles. (ELP; elastic liposomes, RLP; rigid liposomes, CLP; conventional liposome; SUS; drug suspension, SOL; drug solution)

Skin permeation study

The permeation of both lipophilic and hydrophilic drug (MX and NaF) loaded ELP was significantly higher than RLP and CLP as shown in Figure 1D. ELP was able to cross the skin and deliver the drugs (MX and NaF) to the receiver compartment at the high extent, compared to other vesicles and saturated solution of model drugs. The skin permeation profiles are shown in Figure 2. The cumulative skin permeation of ELP was significantly greater than those of CLP, RLP and SUS/SOL, respectively. The ELP has shown to be successful to deliver both lipophilic and hydrophilic drug into the skin, allowing the increased transdermal delivery. The incorporation of edge activator (DCP) might result in the fluidity of the bilayer of ELP, hence ELP could squeeze themselves through the mini-pores between the intercellular lipid in the skin, which are smaller than the vesicles's diameter. Furthermore, DCP was a penetration enhancer that may solubilize the lipid within the stratum corneum and/or interact with the intercellular keratin. [13]

The edge activator and membrane stabilizer affect both the skin permeability (efficacy) and the vesicle stability. Thus the appropriate ratio between DCP and Chol should be considered in the development of liposome formulations for transdermal drug delivery. Chol in the range of 10-40 mol% affects membrane elasticity, rendering the bilayer membrane more rigid. [14] The molar turbidity of ELP decreased markedly at 40 mol% of edge activator. The change in turbidity indicated that ELP vesicles possibly reformed to mixed micelle structure. The different intrinsic properties between ELP and mixed micelle structure may result in different efficacy in the skin permeability. Therefore, the 10-40 mol% edge activator was chosen in this study.

Conclusion

Considering the permeation and skin permeation profiles of the different vesicles, ELP was the optimal vesicle formulations that can be used as transdermal delivery carriers for both lipophilic and hydrophilic drug. The mechanism of action of ELP may attribute to the alteration of the lipid organization and an increase in lipid bilayer disorder. However, the Fourier transform infrared spectroscopy (FT-IR) and the differential scanning calorimetry (DSC) should be used to confirm the disruption of the stratum corneum lipid in further study.

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References

1. Cevc G, Blume G. Biochim Biophys Acta. 1992; 1104 (1): 226-32.
2. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. J Control Release. 2000; 65 (3): 403-18.
3. Song YK, Kim CK. Biomaterials. 2006; 27 (2): 271-80.
4. Dragicevic-Curic N, Gräfe S, Albrecht V, Fahr A. J Photochem Photobiol B. 2008b; 91 (1): 41-50.
5. Dragicevic-Curic N, Scheglmann D, Albrecht V, Fahr A. J Control Release. 2008a; 127 (1): 59-69.
6. Duangjit S, Obata Y, Sano H, Kikuchi S, Onuki Y, Opanasopit P, et al. Biol Pharm Bull. 2012; 35 (10): 1720-8.
7. Duangjit S, Nimcharoenwan T, Chomya N, Locharoenrat N, Ngawhirunpat T. Drug Dev Ind Pharm. 2016: 1-10.
8. Ding WX, Qi XR, Li P, Maitani Y, Nagai T. Int J Pharm. 2005; 300 (1-2): 38-47.
9. Fang JY, Hwang TL, Huang YL, Fang CL. Int J Pharm. 2006; 310 (1-2): 131-8.
10. Rigg PC, Barry BW. J Invest Dermatol. 1990; 94 (2): 235-40.
11. Soema PC, Willems GJ, Jiskoot W, Amorij JP, Kersten GF. Eur J Pharm Biopharm. 2015; 94: 427-35.
12. Fang Y-P, Tsai Y-H, Wu P-C, Huang Y-B. International Journal of Pharmaceutics. 2008; (356): 144–52.
13. Barry BW. Eur J Pharm Sci. 2001; 14 (2): 101-14.
14. Nasseri B. International Journal of Pharmaceutics. 2005; 300 95-101.