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The Role of Deformable Liposome Characteristics on Skin Permeability of Meloxicam: Optimal Transfersome as Transdermal Delivery Carriers

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Abstract: The role of deformable liposomes characteristics on skin permeability has evoked considerable interest, since the articles reporting the effectiveness of transfersomes for skin delivery were increasingly published. Several reports focus on the effect of formulation factor which directly affected the transfersome's skin permeability. However, the effect of formulation factors was not fully understood as the contradictory results. To clarify this problem, the reliable statistical techniques, excellent experimental design and systematical variation were used in this study. Transfersomes loaded meloxicam containing controlled amount of phosphatidylcholine (PC), cholesterol (Chol), type of surfactant (hydrophilic part, lipophilic part) were prepared and investigated for the physicochemical characteristics (e.g., size, size distribution, charge, elasticity, drug content, morphology) and skin permeability. The results indicated that the transfersome containing 10% cholesterol, 29% cetylpyridinium chloride (cationic surfactant) with 16 carbons chain length and 10% meloxicam was the optimal formulation for meloxicam transdermal delivery carrier. The skin permeation of meloxicam in novel transfersome formulation was higher than classical transfersomes, conventional liposomes and meloxicam saturated suspension. Thus, our finding provided important fundamental information for developing novel deformable liposomes for transdermal drug delivery, especially transfersomes containing surfactant systems.

Keywords: Deformable liposomes, transfersomes, liposomes, skin permeation, meloxicam.

1. INTRODUCTION

Meloxicam (MX), a non-steroidal anti-inflammatory drug (NSAID), is used to treat rheumatoid arthritis, osteoarthritis and other joint diseases [1]. The oral administrations of NSAID drugs are very effective, but clinical uses are often limited because of the gastrointestinal (GI) side effects such as peptic irritation and ulceration. Although, MX appears to have partial COX-2 (cyclooxgenease-2) specificity, in practice it still has incidence of GI side effects at high doses on long term therapy [2]. Therefore, the novel pharmaceutical carriers that can modulate GI side effect and deliver MX to the target inflammatory site may prevent or reduce NSAID-induced peptic ulceration.

Liposome system is an alternative carrier to improve drug delivery through the skin. Classical and more recently deformable liposomes have shown their ability to increase dermal and transdermal drug delivery. Several researchers have mentioned that the modification of surface charge of liposomes influences penetration of drug through the skin. It has been reported that the flux of tretinoin using negatively charged liposomes was higher than using positively charged liposomes [3]. On the other hand, numerous studies reported that the flux of various permeants such as low-molecular weight heparin [4], acyclovir [5], minoxidil [5] and temoporfin [6] using positively charged liposomes as carriers was higher than uncharged or negatively charged liposomes. Both consistent and inconsistent results were observed. Our previous study [7] reported that the liposomes can vary with respect to lipid composition. Therefore, the effectiveness of liposomes and their analogues remains a much debated question and has to be tested on a case-by-case basis.

In the development of liposomal drug delivery, the systematic design of the experiment was very important. Our study focuses on the role of surfactant on physicochemical characteristics and skin permeability of meloxicam loaded deformable liposomes. The objectives of the present study were to develop novel transfersomes carriers for transdermal delivery of meloxicam and to investigate the influence of surfactant including hydrophilic part (head group) and lipophilic part (carbon chain). The vesicle size, size distribution, charge, elasticity, drug content in the formulation, skin permeability and skin permeation flux were investigated and compared. A nonlinear response-surface method incorporating thin-plate spline interpolation (RSM-S) was used to estimate the relationships between surfactant type and physicochemical characteristics and skin

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Table 1. The Lipid Compositions of the Different Transfersomes Formulations

	Code	Composition (mM)								
		PC	Chol	SDS (A_{12})	SHS (A_{16})	DPC (C_{12})	HPC (C_{16})	DCP (A_{32})	SA (C_{18})	MX
F1[a]	CLP	10.00	0.10	-	-	-	-	-	-	0.11
F2[b]	aTFS-SDS	10.00	0.10	0.29	-	-	-	-	-	0.11
F3[b]	aTFS-SHS	10.00	0.10	-	0.29	-	-	-	-	0.11
F4[b]	cTFS-DPC	10.00	0.10	-	-	0.29	-	-	-	0.11
F5[b]	cTFS-HPC	10.00	0.10	-	-	-	0.29	-	-	0.11
F6[c]	aTFS-DCP	10.00	0.20	-	-	-	-	0.20	-	0.11
F7[c]	cTFS-SA	10.00	0.20	-	-	-	-	-	0.20	0.11

a; convemtional liposomes, b; novel transfersomes, c; classic transfersomes

permeability. The optimal formulation obtained from RSM-S in our previous study was used as a model formulation.

2. MATERIALS AND METHODS

2.1. Materials

Phosphatidylcholine (PC) was purchased from LIPOID GmbH (Cologne, Germany). Cholesterol (Chol) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Hexadecylpyridinium chloride (HPC) was purchased from MP Biomedicals (Illkirch, France). Sodium hexadecyl sulfates (SHS), sodium dodecyl sulfate (SDS) and dodecylpyridinium chloride (DPC) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Dicetylphosphate (DCP) and stearylamine (SA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Meloxicam (MX) was supplied from Fluka (Buchs, Switzerland). All other chemicals used were of reagent grade and purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Preparation of Meloxicam Loaded Transfersomes

Anionic transfersomes and cationic transfersomes were prepared according to formulations obtained from our previous study [8, 9]. As shown in Table **1**, MX loaded anionic transfersomes and cationic transfersomes composed of a controlled amount of PC, Chol, MX and various types of anionic and cationic surfactant as penetration enhancers were prepared. The amount of PC and MX was fixed at 0.77 and 0.07 (%w/v), respectively. Liposome and transfersome formulations were prepared by sonication method. Briefly, the lipid mixtures of PC, MX, Chol and surfactant were dissolved in chloroform/methanol (2:1 v/v ratio). These lipid mixtures were mixed and evaporated under nitrogen gas stream. The lipid film was placed in a desiccator for at least 6 h to remove the remaining solvent. The dried lipid film was hydrated with the acetate buffer solution (pH 5.5). Liposome and transfersome formulations were subsequently sonicated for 2 cycles of 15 min. using a bath-type sonicator (5510J-DTH Branson Ultrasonics, CL, USA). Liposome and transfersome formulations were freshly prepared or stored in airtight container at 4 ºC prior to use. Moreover, the MX-suspension (SUS) was prepared by incorporating 2 times of MX higher than its solubility in acetate buffer solution (pH 5.5) and stirring for 24 h to ensure constant thermodynamic activity through the course of the skin permeation experiment.

2.3. Measurement of Vesicles Size, Size Distribution, Zeta Potential and Morphology

The vesicle size, size distribution and zeta potential of liposome and transfersome formulations were measured by photon correlation spectroscopy (Zetasizer Nano series, Malvern Instrument, UK). Twenty µl of the vesicle formulations were diluted with 1480 µl of deionized water. All measurement samples were performed at least three independent samples were taken, and the vesicle size, size distribution and zeta potential were measured at least three times, at room temperature.

The morphology of the transfersomes was observed by freeze-fractured electron microscopy. A small drop of sample solution placed on a small copper block was rapidly frozen in nitrogen slash, which was freshly prepared just before its use by decompression in a vacuum chamber [10]. The quenched sample was fractured in a freeze-fractured apparatus JFD-9010 (JEOL, Tokyo, Japan). The fractured surface was rotary-shadowed with platinum-carbon at an angle of 10º and the shadowed surface was coated with carbon. The freeze-fractured replica obtained was washed with chloroform/methanol (4:1 v/v ratio) and observed with a transmission electron microscope JEM1400 (JEOL, Tokyo, Japan) equipped with a digital CCD camera (ES500W Erlangshen, Gatan, USA).

2.4. Measurement of Elasticity Value

The elasticity value of lipid bilayers of the vesicles was directly proportional to J_{Flux} x $(r_v/r_p)^2$;

$$\text{Elasticity value} = J_{Flux} \times (r_v/r_p)^2$$

where J_{Flux} is the rate of penetration through a permeable barrier ($mg \cdot sec^{-1} \cdot cm^{-2}$), r_v is the size of the liposomes or transfersomes after extrusion (nm) and r_p is the pore size of the barrier (nm) [11]. To measure J_{Flux}, the vesicles were extruded through a polycarbonate membrane (Nuclepore, Whatman Inc., MA, USA) with a pore diameter of 50 nm

(r_p), at a pressure of 0.5 MPa. After 5 min of extrusion, the extrudate was weighed (J_{Flux}), and the mean of vesicle diameter after extrusion (r_v) was determined by the photon correlation spectroscopy at room temperature.

2.5. Determination of MX in the Formulation

The concentration of MX in the formulation was determined by HPLC analysis after disruption of the liposomes or transfersomes 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 vesicle/Triton® X-100 solution was centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was filtered with a 0.45 μm nylon syringe filter.

2.6. *In vitro* Skin Permeation Study

The excised skin of hairless mice (Laboskin®, HOS: HR-1 Male, 7 weeks, Sankyo Labo Service Corporation, INC., Tokyo, Japan) was used as a skin model for the *in vitro* skin permeation study. A side-by-side diffusion cell with an available diffusion area of 0.95 cm^2 was employed. The receiving chamber was filled with 3 ml of phosphate buffer solution (pH 7.4, 32 ºC) and the donor chamber was filled with 3 ml MX loaded anionic transfersomes (aTFS), cationic transfersomes (cTFS), conventional liposomes (CLP) or MX-suspension (SUS). At appropriate times, an aliquot of the receiver fluid was withdrawn and the same volume of fresh buffer solution was replaced to the receiver chamber. The concentration of MX in the aliquot was analyzed using an HPLC.

2.7. HPLC Analysis of Meloxicam

The HPLC system consists of a SIL-20A autosampler, LC-20AT liquid chromatography and SPD-20AUV detector (Shimadzu Corporation, Kyoto, Japan). The analytical column was YMC-Pack ODS-A (150 mm × 4.6 mm i.d., S-5, YMC Co. Ltd, Kyoto, Japan), and the mobile phase was composed of acetate buffer solution (pH 4.6)/methanol (50:50, v/v). The flow rate was set at 0.8 ml/min, and the wavelength used was 272 nm. The calibration curve for MX was in the range of 1-50 µg/mL with a correlation coefficient of 0.999. The percentage recovery was found from 99.57-100.30%, and relative standard deviation for both intra-day and inter-day was less than 2%.

2.8. Data Analysis

The data were reported as mean ± S.E. (n=3-6) and statistical analysis of the data was carried out using one way ANOVA followed by LSD *post hoc* test. A *p*-value of less than 0.05 was considered to be significant.

2.9. Ethics in the Animal Study

This animal study was performed at Hoshi University and complied with the regulations of the committee on Ethics in the Care and Use of Laboratory Animals.

3. RESULTS

Based on the optimal formulation obtained from RSM-S in our previous study, different meloxicam loaded transfersomes formulations were formulated in order to investigate the influence of surfactant i.e., charge (cationic, anionic) and carbon chain (medium chain, long chain) on physicochemical characteristics and skin permeability of liposomes and transfersome formulations.

3.1 The Physicochemical Characteristics of Liposomes and Transfersomes Formulation

The physicochemical characteristics of conventional liposomes (CLP), anionic transfersome (aTFS), cationic transfersomes (cTFS) and classic transfersomes were investigated and compared. As shown in Fig. (**1**), the vesicle size was between 80 and 200 nm. The polydispersity index of all formulations was between 0.2 and 0.4. The charge of different formulation depended on their liposomes type. The negative charge was observed in aTFS, whereas the positive charge was observed in cTFS. However, no any charge was observed in CLP. The elasticity of different formulation depended on type of lipid composition in liposomes. Both aTFS and cTFS had higher elasticity index than CLP. The drug content in the different formulation was 170-520 µg/mL.

The morphology of liposomes and transfersomes observed under freeze-fracture transmission electron microscopy was sphere shape and smooth surface. As shown in Fig. (**2**), the size of both vesicles was around 100 nm under the electron microscopy.

3.2 The skin permeation study of the different formulations

Fig. (**3**) shows the skin permeation profile of cTFS, aTFS, CLP and meloxicam suspension (SUS). The amount of meloxicam permeation of liposome and transfersome formulation was significantly higher than meloxicam suspension. Furthermore, the amount of meloxicam permeation of both charges of TFS was also significantly higher than CLP. Fig. (**4**) shows the skin permeation flux of control, aTFS, cTFS and classic TFS. The skin permeation flux of cTFS and aTFS was significantly higher than classic TFS, CLP and SUS.

4. DISCUSSION

The addition of anionic surfactant in liposomes significantly increased vesicle size, negative charge, elasticity and drug content in the formulation. The intensive properties of anionic surfactant could increase the negative charge of meloxicam loaded aTFS. Moreover, anionic surfactant could increase the repulsive force between intrabilayer of aTFS, thus resulting in an increase the vesicle size [12]. The head group of anionic surfactant may affect the net negative charge of aTFS. The anionic surfactant could increase the elasticity of aTFS by destabilizing the bilayer [13]. Moreover, it may act as solubilizing agent in bilayer, thus aTFS showed an increase in the drug content.

The intensive properties of cationic and anionic surfactant were contrast, thus the effect of cationic surfactant on physicochemical characteristics of cationic transfersomes (cTFS) was significantly different. The addition of cationic surfactant in liposomes slightly decreases vesicle size and significantly increases positive charge of cTFS. The neutralization of negative charge of meloxicam and positive charge of vesicle may result in a decrease in the repulsive force between intrabilayer, therefore resulting in a decrease

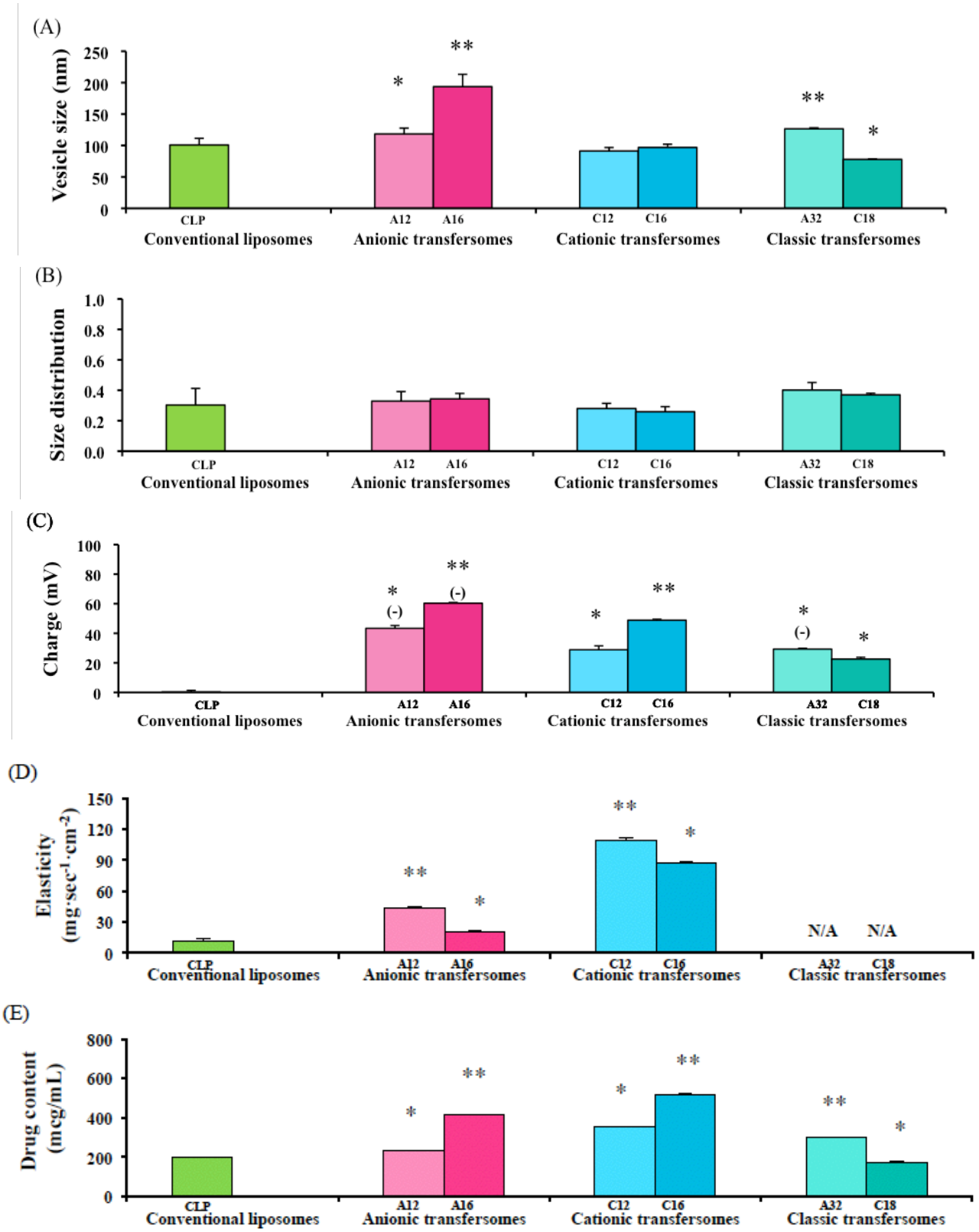

Fig. (1). The physicochemical characteristics of the different formulation: (**A**) size, (**B**) size distribution, (**C**) charge, (**D**) elasticity and (**E**) drug content (* p <0.05, compared with conventional liposomes and ** p <0.05, compared within the same group of transfersomes).

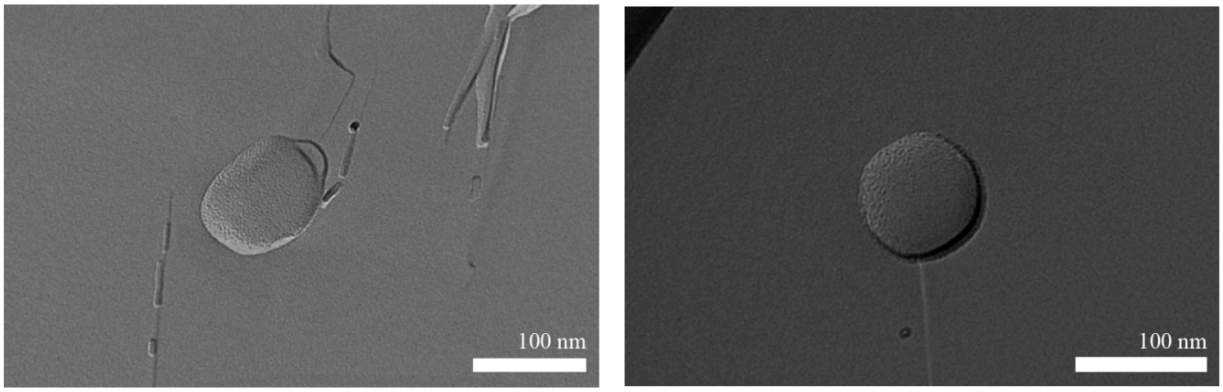

Fig. (2). The morphology of liposomes (left) and transfersomes (right) under freeze-fracture transmission electron microscopy

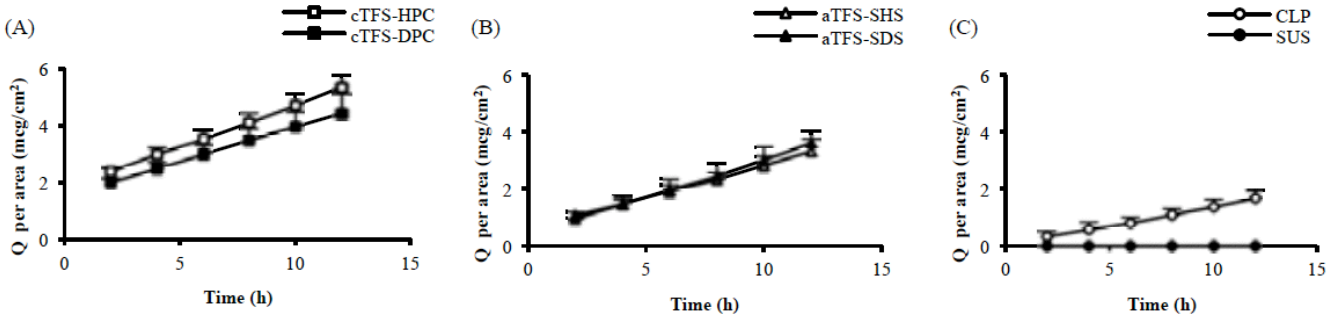

Fig. (3). The skin permeation profile of the different formulation: (**A**) control (**B**) anionic transfersomes and (**C**) cationic transfersomes

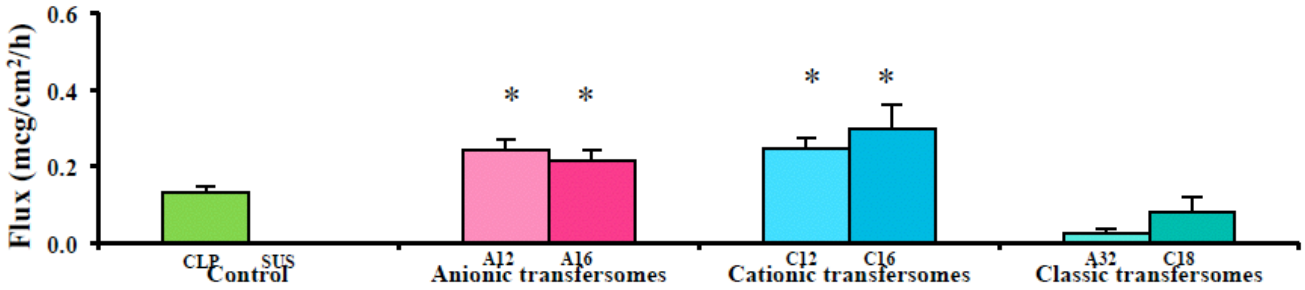

Fig. (4). The skin permeation flux of the different formulation (* p <0.05, compared with conventional liposomes)

in vesicle size of cTFS [12]. While the head group of cationic surfactant may affect the net positive charge of cTFS, the cationic surfactant could also increase the elasticity and increase the drug content of cTFS by destabilizing the bilayer and acting as solubilizing agent in bilayer, respectively.

The effect of carbon chain of both surfactants (anionic and cationic) on physicochemical characteristics of transfersomes was in the same trend. As carbon chain of surfactant was increased, the charge also increased. The bilayer is composed of PC containing one saturated fatty acid (C16) and one unsaturated fatty acid (C18). The long chain carbon may have electrostatic interaction with bilayer stronger than short chain carbon, resulting in an increase of charge and drug content of both aTFS and cTFS [14]. While the lipophilic properties of long chain carbon was stronger than short chain, therefore long chain may deeply insert and becomes tighter, thus resulting in a decrease in elasticity [15].

The amount of meloxicam permeated through the skin of cTFS was significantly higher than aTFS and classic transfersomes. The physicochemical characteristics of cTFS, aTFS and classic transfersomes was significantly different, therefore skin permeability of meloxicam in cTFS was greater than other formulations. The net positive charge of the vesicle might interact with the negative charge of the skin, thus the skin permeation of meloxicam cTFS increased. The high elasticity and high drug content may be the main factor affecting the skin permeation of meloxicam loaded cTFS. The possible mechanisms of meloxicam loaded cTFS could be explained by the penetration enhancing mechanism, the vesicle adsorption to or fusion with the stratum corneum and/or the intact vesicular skin permeation mechanism [16].

However, the surfactants used were different in various points such as charge, carbon chain length and structure of polar moiety. The change of these factors might be difficult to separate the effect of one factor from other factors. Therefore, it is necessary to concern about systematic sample preparation. The effect of cholesterol, critical micellar concentration and polar head group of surfactant (critical packing parameter and interaction in the interfacial region) should be considered on the bilayer characteristics.

5. CONCLUSIONS

The skin permeation of meloxicam through hairless mice skin of our novel transfersome formulation was significantly higher than classical transfersomes, conventional liposomes and meloxicam saturated suspension. Thus, our finding provided important fundamental information for developing novel transfersomes for transdermal drug delivery.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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DISCLOSURE

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