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Triamcinolone permeation from different liposome formulations through rat skin in vitro

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Abstract

The permeation of triamcinolone acetonide (TRMA) from various liposome formulations through rat skin was studied in vitro. The penetrated amount, permeability and intradermal retention of TRMA were compared among various lipid compositions, different vesicle sizes (0.2, 0.4 and 1 μm), charges (positive, negative and neutral), as well as between multilamellar vesicles (MLV) and small unilamellar vesicles (SUV). All of the liposome formulations resulted in significantly higher flux and permeability of TRMA than a commercial TRMA ointment. The 'skin lipid' liposome provided the most effective transdermal delivery of incorporated TRMA. Presence or absence of cholesterol in the lipid bilayers did not reveal any difference in transdermal delivery of the associated TRMA. The flux and permeability of TRMA through skin were not influenced by the vesicle size of MLV, but was significantly increased by negative SUV. Intradermal retention of TRMA from positive MLV was significantly higher, while that from neutral SUV was significantly lower, than from other formulations. Liposomal lipid was not detectable on the receptor compartment. These results suggest that liposome itself may not penetrate through the skin, but that it does enhance the transfer of incorporated TRMA. Liposomal lipid composition is the most important factor affecting the efficiency of transdermal delivery of incorporated drugs, but was not correlated with its phase transition temperature.

Keywords: Liposomes; Triamcinolone; Skin permeation; Transdermal; Liposome surface charge

1. Introduction

Liposomes have shown great potential as novel carriers for dermal and transdermal systems. Liposomes can increase the permeability of the skin for various compounds (Mezei and Gulasekharam, 1980; Ganesan et al., 1984). In the field of cosmetics (Hayward and Smith, 1990; Suzuki and Sakon, 1990) liposomes are believed to increase the barrier function of the skin and decrease water loss within a short period of time after application (Prottey et al., 1975; Tieger, 1987). Successful laboratory experiments with topical applications of liposomes as drug localizers in microsurgery have been reported (Hou et

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al., 1991). Although the use of liposomal formulations for topical application has been steadily increasing, little work has been done on skin permeation of the liposomal system.

The topical application of glucocorticoids in dermatology has recently become quite common. Mezei and Gulasekharam (Mezei and Gulasekharam, 1980, 1982) have reported that topical application of triamcinolone acetonide (TRMA) liposome increased the epidermic concentration and decreased systemic absorption of TRMA when compared with TRMA ointment. However, only one formulation of TRMA liposome was presented in their report. The pharmacokinetics of liposome preparations can be affected by changes in formulations and process of preparation, such as lipid composition, liposome surface charge, vesicle type and size. In addition, whether or not phospholipid molecules are able to penetrate through the skin is still controversial; positive (Abraham and Downing, 1990) and negative (Knepp et al., 1988) results have been reported.

The objective of this study was to investigate the effects of liposomal formulations on skin permeation of TRMA. The main differences between the formulations was the lipid composition, the surface charge, as well as the vesicle types of liposomes. The results were compared with commercial TRMA ointment. The mechanism for the transport of entrapped drug was also examined.

2. Materials and methods

2.1. Chemicals

Lipids for preparation of liposomes were purchased from Sigma (St. Louis, MO, USA), [^{14}C]cholesterol and [^{3}H]triamcinolone acetonide from Du Pont (USA), other reagents from E. Merck (Germany). TRMA crystalline powder and ointment (0.1%) were generous gifts from Bristol and Squibb (Taiwan branch).

2.2. Preparation of TRMA liposomes

Liposomes containing TRMA 0.1% and lipid constituents 100 mg/ml were prepared. Various liposomal lipid formulations are listed in Table 1. The lipid mixture containing TRMA (cold with a trace of [^{3}H]TRMA) was dissolved in chloroform and evaporated under a stream of purified nitrogen in the slowly rotated round bottom flask of a rotatory evaporator until a thin film of the lipid mixture was formed in the inner wall of the flask. Traces of solvent which remained in the lipid film were removed under overnight vacuum desiccation. Phosphate buffer saline (10 mM, pH 7.2, Sigma) was added to the dried lipid film and then shaken on a vortex mixer to produce multilamellar vesicles (MLVs). The liposome suspension was then extruded through a polycarbonate membrane (Nucleopore Co., USA), by nitrogen pressure, for sizing. The diameter of liposomes was measured by a photal particle counter (LPA-3000, Otsuka, Japan).

The small unilamellar vesicles (SUV) were prepared by intermittent sonication of MLV under 10 amplitude micron power in a probe type sonicator (Soniprep 150, MSE, UK) for 60 min.

2.3. Permeation measurements with rat skin

Wistar rat skin was used. The rats (body weight about 300 g) were sacrificed by ether inhalation; the hair was shaved and the skin cut to a size adequate for permeation studies. The permeation study was carried out using the Franz-type diffusion cell (exposed skin diameter 1 cm) kept at 37°C in a water bath. In the receptor compartment was phosphate buffer solution (pH 7.2),

Table 1
Lipid constituents of the liposomes

Neutral (o) liposomes	PC:CH = 2:1	m/m
Negative (−) liposomes	PC:CH:DP = 2:1:0.25	m/m
Positive (+) liposomes	PC:CH:SA = 2:1:0.25	m/m
DPPC liposomes	DPPC	
PC liposomes	PC	
PC/CH liposomes	PC:CH = 1:1	
Skin liposomes [16]	CA:CH:PA:CS = 4:2:2.5:1	w/w

CA, ceramide; CH, cholesterol; CS, cholesteryl sulfate; DP, dicetylphosphate; DPPC, dipalmitoyl phosphatidylcholine; PA, palmitic acid; PC, egg L-α-phosphatidylcholine; SA, stearylamine; m/m, molar ratio; w/w, weight ratio.In the parentheses (o, − and +) are symbols of charges.

Table 2
Skin-permeation and retention of TRMA and liposomal lipids after applying for 45 h in vitro

		TRMA			Lipid	
		Penetrated amount (% Dose)	Flux (mg/cm^2 per h)	Retention (% Dose)	Penetrated amount (% Dose)	Retention (% Dose)
o MLV		1.9 ± 0.3	1.2 ± 0.2	0.55 ± 0.04	0	0.14 ± 0.01
o SUV		1.5 ± 0.2	0.86 ± 0.01	$0.31 \pm 0.02^{a,b}$	-	
+ MLV		1.9 ± 0.5	1.4 ± 0.5	1.4 ± 0.3^{b}	0	0.23 ± 0.03
+ SUV		1.9 ± 0.2	1.2 ± 0.08	0.66 ± 0.04	0	0.18 ± 0.02
− MLV						
	1 μm	1.0 ± 0.1	0.70 ± 0.06	0.46 ± 0.08	0	0.07 ± 0.01
	0.4 μm	0.96 ± 0.12	0.68 ± 0.08	0.40 ± 0.05	0	0.10 ± 0.02
	0.2 μm	1.1 ± 0.2	0.68 ± 0.09	0.34 ± 0.07	0	0.07 ± 0.01
− SUV		2.3 ± 0.4^{a}	1.4 ± 0.2^{a}	0.59 ± 0.05	0	0.22 ± 0.11
PC[f]SUV		1.6 ± 0.2	1.0 ± 0.1	0.23 ± 0.03	-	-
DPPC SUV		1.0 ± 0.08^{c}	0.64 ± 0.05^{c}	0.17 ± 0.02	-	-
Skin SUV		7.2 ± 0.4^{d}	4.7 ± 0.2^{d}	1.30 ± 0.06^{d}	0	0.54 ± 0.07
Ointment		0.37 ± 0.03^{e}	0.25 ± 0.02^{e}	0.19 ± 0.03^{e}	-	-

Data are means ± S.E. of five to six experiments.-, not determined.[a]Significantly different ($P < 0.05$), comparing between MLV and SUV of the same charge.[b]Significantly different ($P < 0.05$), comparing among the same vesicle type bearing different charge.[c]$P < 0.05$, compared with PC.[d]$P < 0.01$, compared with PC and DPPC.[e]Significantly lower ($P < 0.01$) than liposome formulations.[f]Results are statistically not different from PC/CH formulation (neutral SUV).

containing 0.02% streptomycin and 0.012% penicillin G, to prevent skin deterioration during the experiment period. On a time schedule up to 45 h, an aliquot of 200 μl from the receptor compartment was sampled and replaced by an equal volume of fresh phosphate buffer saline.

The amount of [^{3}H]TRMA and [^{14}C]cholesterol retained in the applied skin and in the receptor compartment was determined by a liquid scintillation counter (Beckman, LS-6001, USA).

2.4. Calculation

The cumulative amount of TRMA presented in the receptor compartment during the nth sampling (Q_n) was estimated by Eq. (1):

$$Q_n = C_n \times V + V_s \times \sum_{i=1}^{n-1} C_i \quad (1)$$

where C is the measured concentration in the nth sample, V is the volume of receptor solution, and V_s is the volume of sampling. Fluxes (J) were determined from the slope of the cumulative amount of penetrated TRMA versus time (t), and permeability coefficient (P) was estimated according to Eq. (2) based on the fact that drug concentration in the receptor compartment is negligible compared with that in the donor compartment (C_d).

$$P = J/C_d \quad (2)$$

Student's t-test was used to compare two groups. One-way analysis of variance (ANOVA) was used to test the significance of differences among three groups, and Turkey's test was used when necessary, to further determine which groups differed from the others. Significance was set at $P < 0.05$.

3. Results

The skin permeation and retention of TRMA and liposomal lipid from various liposome formulations are summarized in Table 2 and Fig. 1. Linearities of TRMA flux versus time were observed implying that the concentration gradient in the donor compartment is negligible.

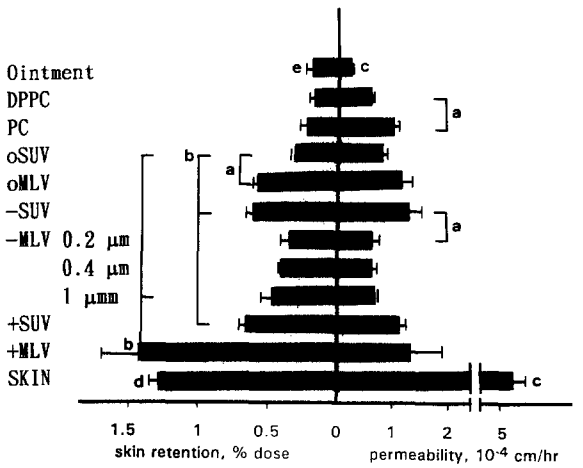

Fig. 1. Retention and permeability of TRMA from various formulations. Key: significantly different: a, between the two; b, to the other two of the same charge, c, from all the others; d, from all the others except + MLV; e, from all the MLV, − SUV and SKIN.

3.1. Effects of vesicle sizes

A homogeneous liposome suspension with a narrow size distribution was obtained after passing the liposomes through polycarbonate membrane or by sonication (Table 3). The liposome vesicle size was almost equivalent to the membrane pore diameter. Sonication produced SUV of PC around 0.03 μm, of dipalmitoyl phosphatidylcholine (DPPC) around 0.08 μm, and of skin lipid about 0.16 μm.

Table 3
Size distribution of liposomal vesicles after extrusion through polycarbonate membrane or sonication

Membrane pore (μm)	Liposomes	Vesicle diameter (μm)
MLVs		
1.0	Neutral	0.95 ± 0.061
	Positive	0.80 ± 0.052
	Negative	0.96 ± 0.062
0.4	Negative	0.40 ± 0.047
0.2	Negative	0.21 ± 0.025
SUVs		
	Negative	0.035 ± 0.004
	Neutral	0.032 ± 0.004
	Positive	0.027 ± 0.003
	PC	0.031 ± 0.004
	DPPC	0.076 ± 0.011
	Skin	0.161 ± 0.064

Data are means ± S.D.

The size of TRMA MLVs did not show significant difference in permeation and retention of TRMA to the applied skin (Table 2).

3.2. Effects of vesicle types

Comparing between MLV and SUV within same vesicle charge, the negative SUV gave a significantly higher penetrated amount and permeability, and the neutral SUV revealed a significantly lower skin retention of TRMA, than the corresponding MLVs of the same charge.

3.3. Effects of vesicle charges

Comparing different surface charges among the same vesicle type (MLVs or SUVs), the penetrated amount and permeability of TRMA was not statistically different, but the skin retention of TRMA was different. The positive MLV showed significantly higher, and the neutral SUV showed a significantly lower, skin retention of the active ingredient than others of the same vesicle type.

3.4. Effects of lipid composition

Lipid compositions of liposomes influenced the transdermal activities of entrapped TRMA. TRMA in the PC liposome showed a significantly higher penetrated amount and permeability of TRMA than that in the DPPC liposome. Liposomes consisting of skin lipid composition more significantly enhanced the skin permeation of TRMA than other liposomes (Fig. 1). Skin retention of TRMA from skin lipid liposome was comparable with that from positive MLV, and was significantly higher than that from other liposome formulations. Neutral liposome formulation devoid of cholesterol (i.e. PC liposome) did not show significant alterations in skin permeation and retention of TRMA.

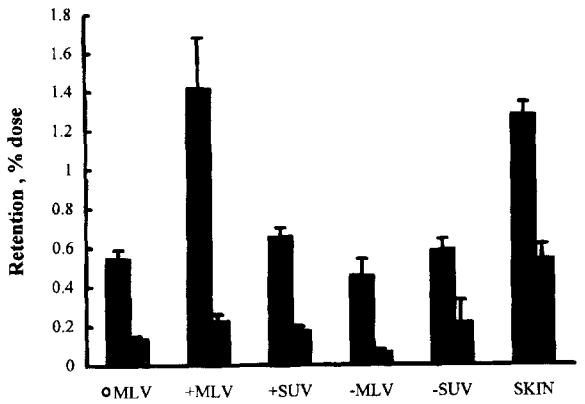

Fig. 2. Retention of TRMA (gray) and liposomal lipid (black) in skin after applying liposome formulations for 45 h.

3.5. Comparing with ointment

The TRMA liposomes showed a significantly improved transdermal activity and skin retention of the active ingredient than the TRMA ointment did (Table 2 and Fig. 1).

3.6. Liposome–skin interaction

Liposomal lipid was not detectable in the receptor compartment, and only a trace of lipid, much less than the component TRMA, was found in the skin after application for 45 h (Table 2 and Fig. 2).

4. Discussion

TRMA, very slightly soluble in water, is intercalated in the lipid bilayers of the liposome membrane but not in the enclosed aqueous space. There was no detectable TRMA in the aqueous phase. Consequently, the entrapment efficiency and the stability in terms of drug leakage are not problems for TRMA liposomes.

The liposomal membrane charges of TRMA liposomes did not show differences in skin permeation of TRMA but revealed differences in intradermal retention (Fig. 1). The TRMA in positive MLVs showed a significantly higher intradermal retention of TRMA than did other phospholipid formulations (TRMA in positive SUVs also showed higher intradermal retention but failed to reach statistical significance). This result was in agreement with a report that the corneal uptake of liposome-associated radioactivity was found to be greatest for positively charged liposomes (Taylor et al., 1982), because at physiological pH the cell surface bears a net negative charge.

Cholesterol can alter the fluidity of the liposomal membrane both above and below the phase transition temperature (T_c): it decreases the fluidity above this temperature, and increases the fluidity below this temperature (New, 1990). To investigate whether fluidity of liposomal membrane is responsible for the differences observed from different lipid compositions, TRMA liposomes consisting of phosphatidylcholine (T_c, -7°C) (Chapman, 1984), with or without cholesterol were compared. The results (Table 2) suggest that fluidity of the lipid components may be not an important factor for determining permeation and retention of the entrapped TRMA, and the higher TRMA permeation from PC (T_c -7°C) than DPPC (T_c 41°C) (Chapman, 1984) may not be due to better fluidity of PC. This finding is further supported by the result observed that skin lipids, with higher T_c (87.9°C) than PC and DPPC, showed the highest skin permeation and retention of TRMA. The 'skin lipids mixture', the composition of stratum corneum lipids, does not contain phospholipids. Nevertheless, the skin lipid mixture has been proved to form bilayers as well as SUV at physiological pH (Wertz et al., 1986). Among the formulations studied, TRMA in skin lipid liposome showed the highest efficiency for TRMA transport through the skin. The best result exerted by the skin-lipid liposome might have come from its optimum miscibility with the lipid layer of the skin, and thus might facilitate the release of TRMA from formulation and transport of TRMA through skin.

It is evident that liposomal TRMA formulations are superior to conventional ointment formulations in facilitating drug retention in, and permeation through rat skin. The permeability of TRMA in any liposome formulation was higher than conventional ointment formulation. Liposomal lipid was not found in the receiving compart-

ment. This result is consistent with a study using [^{14}C]phospholipid as a tracer (Ganesan et al., 1984). The results indicated that a whole liposome vesicle does not penetrate intact, through the skin at all, but merely facilitates transfer of TRMA having been incorporated in liposomal bilayers through the skin. Phospholipids possess the property of surfactants which are thought to exert a 'pull' effect on the membrane (Kadir et al., 1987), i.e. to penetrate into the intercellular lipid bilayers (where the barrier function of the skin is located), thereby reducing the crystallinity of the intercellular lipid bilayers and thus increasing the permeability of these bilayers. TRMA is very slightly soluble in water. In spite of no measurable amount of TRMA being found in the aqueous phase of the liposome preparation, cumulative TRMA was present in the receptor buffer solution. The same phenomenon has been observed in a study of progesterone (Ganesan et al., 1984). These results further support the hypothesis that the TRMA intercalated in the liposomal bilayer might initially be released into the skin and then transfer from skin to the receptor compartment, i.e. the partition of TRMA may not between liposomal lipid and aqueous solution but between skin and its surroundings.

The present in vitro study demonstrated that lipsomes can increase intradermal concentration and thus facilitate the transdermal efficiency of a water insoluble drug carried by the liposomes. The partitions of TRMA and liposomal lipids from viable skin to adjacent tissues might be higher than that observed from in vitro study owing to the more lipophilic property of tissue (in vivo) than buffer solution (in vitro) on the receptor side. However, systemic absorption of a whole liposome vesicle from an externally applied liposome preparation would not take place unless the vesicle was small enough and could pass without disintegration through the intercellular junctions of the skin and tissues. The lipase activity in viable skin, which may cause the degradation of liposomal vesicle lipids, may also make the skin retention of liposomal TRMA in vivo somewhat different from the present in vitro study.

The pronounced higher skin retention of TRMA from both positive and skin lipid liposomes implies that the skin-formulation interactions are not a mere absorption, which is favorable for positive liposomes, but that an exchange of material between liposomes and skin, which is favorable for skin lipid liposomes, may take place.

On the basis of data presented herein, the liposomal dosage form is superior to the ointment form in terms of topical drug delivery. Lipid constituents of liposomes showed more pronounced influence than size or charge of liposomes to skin permeation and localization of triamcinolone, but the phase transition temperature of the lipid was not a critical factor. Skin lipid liposomes greatly enhance skin permeation and localization of triamcinolone.

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