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Enhancement of follicular delivery of finasteride by liposomes and niosomes 1. *In vitro* permeation and *in vivo* deposition studies using hamster flank and ear models

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Abstract

Finasteride is indicated orally in the treatment of androgenetic alopecia and some other pilosebaceous unit (PSU) disorders. We wished to investigate whether topical application of finasteride-containing vesicles (liposomes and niosomes) could enhance drug concentration at the PSU, as compared to finasteride hydroalcoholic solution (HA). Liposomes consisted of phospholipid (dimyristoyl phosphatidylcholine (DMPC) or egg lecithin):cholesterol:dicetylphosphate (8:2:1, mole ratio). Niosomes were comprising non-ionic surfactant (polyoxyethylene alkyl ethers (Brij® series) or sorbitan monopalmitate):cholesterol:dicetylphosphate (7:3:1, mole ratio). Vesicles were prepared by the film hydration technique and characterized with regard to the size, drug entrapment efficiency and gel–liquid transition temperature (T_c). *In vitro* permeation of ^{3}H-finasteride through hamster flank skin was faster from hydroalcoholic solution (0.13 μg/cm^2 h) compared to vesicles (0.025–0.058 μg/cm^2 h). *In vivo* deposition of ^{3}H-finasteride vesicles in hamster ear showed that liquid-state vesicle, i.e. those made of DMPC or Brij97:Brij76 (1:1), were able to deposit 2.1 or 2.3% of the applied dose to the PSU, respectively. This was significantly higher than drug deposition by gel-state vesicles (0.35–0.51%) or HA (0.76%). Both *in vitro* permeation and *in vivo* deposition studies, demonstrated the potentials of liquid-state liposomes and niosomes for successful delivery of finasteride to the PSU.

Keywords: Finasteride deposition in the pilosebaceous units; Niosomes; Liposomes; Skin permeation; Hamster; Follicular drug delivery

1. Introduction

Skin disorders such as acne, seborrhea, hirsutism and androgenetic alopecia are secondary to excess local activity of androgens, more specifically dihydrotestosterone (DHT) in the pilosebaceous unit (PSU). DHT is formed from testosterone via the pivotal enzyme, 5α-reductase. The possibility to target 5α-reductase inhibitors to the PSU to alleviate the disease states associated with or originating within PSU has been implicated in a number of studies (Matias and Orentreich, 1983; Matias et al., 1988; Lieb et al., 1994; Chen et al., 1995).

Finasteride is a known 5α-reductase inhibitor (Tian et al., 1994; Bull et al., 1996) expected to be pharmacologically active in the pilosebaceous unit (Chen et al., 1995, 1996). There have been several clinical studies on the efficacy of oral finasteride in the treatment of seborrhoea (Chen et al., 1996), female hirsutism (Moghetti et al., 1994; Wong et al., 1995) and androgenetic alopecia (Dallob et al., 1994; Chen et al., 1996; Kaufman et al., 1998; Drake et al., 1999; Meidan and Touitou, 2001; Shapiro and Kaufman, 2003). Finasteride also has been used topically to treat androgenetic alopecia (Mazzarella et al., 1997).

In recent years, many attempts have been made to enhance drug deposition in the PSU using delivery systems such as nanoemulsions (Lieb et al., 1992; Wu et al., 2001), solid lipid nanoparticles (Maia et al., 2002), low-molecular weight dextrans (Lieb et al., 1995), microspheres (Rolland et al., 1993; Sumian et al., 1999; Toll et al., 2004), iontophoresis (Burnette and Ongpipattanakul, 1988; Cullander and Guy, 1991; Green

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et al., 1991) and niosomes or liposomes (Balsari et al., 1994; Lieb et al., 1994; Lauer et al., 1995, 1996; Niemiec et al., 1997; Bernard et al., 1997; Weiner, 1998; Toll et al., 2004). Experiments with liposomes or niosomes in the Syrian hamster ear model have demonstrated that the vesicles containing carboxyfluorescein (Lieb et al., 1992), cimetidine (Lieb et al., 1994) and peptides (Niemiec et al., 1995) delivered higher drug concentrations into the sebaceous glands as compared to conventional formulations. High drug localization into the hair follicles of histocultured mouse have been also reported for calcein (Li et al., 1992), melanin (Li et al., 1993b) and DNA (Li et al., 1993a) containing liposomes.

Liposomes and niosomes, made of phospholipids and non-ionic surfactants, respectively, have several advantages over conventional non-vesicle formulations. A major advantage lies in their amphipathic nature, which allows incorporation of a wide variety of hydrophilic and hydrophobic drugs (Uchegbu and Vyas, 1998). They may serve as a solubilizing matrix, as a local depot for sustained release or permeation enhancers of dermally active compounds or as a rate limiting membrane for the modulation of systemic absorption of drugs via the skin (Touitou et al., 1994; Agarwal et al., 2001; Manosroi et al., 2003).

Liposome and niosome characteristics such as lamellarity, lipid composition and structure (El Maghraby et al., 1999), surface charge and size (Plessis et al., 1994), as well as physicochemical nature of the drug itself (Lauer, 1999; Grams and Bouwstra, 2002; Ogiso et al., 2002; Grams et al., 2003) may affect of follicular deposition of drugs. Vesicle–skin interactions and the therapeutic efficacy of vesicular formulation can be strongly affected, in particular, by phase state (Bouwstra et al., 1997; Uchegbu and Vyas, 1998) and elasticity (van den Bergh et al., 1999; El Maghraby et al., 1999; Trotta et al., 2002); bilayers in the liquid-state are not closely packed as in the gel state, and thus can penetrate within skin strata more easily.

In this study, we sought to evaluate the effects of composition and physical state of vesicles on the extent of finasteride permeation through and deposition into the different strata of the hamster flank and ear skin.

2. Materials and methods

2.1. Materials

Non-ionic surfactants: Brij52 (polyoxyethylene 2 cetyl ether), Brij72 (polyoxyethylene 2 stearyl ether), Brij76 (polyoxyethylene 10 stearyl ether), Brij97 (polyoxyethylene 10 oleyl ether), Span40 (sorbitan monopalmitate) and cholesterol (chol) were purchased from Fluka (Switzerland). Dicetyl phosphate (DCP) was obtained from sigma (USA). Dimyristoyl phosphatidylcholine (DMPC) was from Nippon Fine Chemicals (Japan). ^{3}H-finasteride, having the specific activity of 25 Ci/mmol, was purchased from American Radiochemicals (ARC, USA) and finasteride was from Cipla Inc. (India). Clobazam was kindly provided by Hakim Pharmaceuticals (Tehran, Iran). Hyamine hydroxide (ICN Biochemical) was used as a tissue solubilizer. Ready Value®, Ready organic® and Ready Protein+® scintillation cocktail were obtained from Beckman (USA). Sephadex G-25 coarse was bought from Pharmacia LKB (Uppsala, Sweden). Egg lecithin, acetonitrile LC grade and methanol LC grade were obtained from Merck (Germany). All other chemicals and solvents were of analytical grade. HPLC-grade de-ionised water was produced using Direct-Q^{TM} (Millipore, France).

2.2. Animals

Male adult Golden Syrian hamsters (weighing 80–110 g), 10–12 weeks of age, were purchased from Pasteur Institute (Tehran, Iran). The animals were housed one per cage in plastic boxes on sawdust with tap water. The animals were housed at a photoperiod of about 14-h light and 10-h darkness and ambient temperature, for at least 2 weeks prior to the experiments. The skin overlying the flank organs was closely shaved with an electric hair clipper. The experiments using animals were approved by the university's ethics committee.

2.3. Vesicle preparation

Multilamellar vesicles (MLVs) were prepared by the film formation method as reported by Bangham et al. (1965) and Baillie et al. (1985) with some modifications. Lipid components of niosomes comprising surfactant (one or mixture of two):chol:DCP (7:3:1, m.r.) or liposomes consisting phospholipid (egg lecithin or DMPC):chol:DCP (8:2:1, m.r.) were dissolved in chloroform:methanol (2:1 v/v). The total lipid concentration was 30 mM. Finasteride solution in the same solvent (0.53 mM) was spiked with ^{3}H-finasteride at 8 μCi/ml and added to the lipid solution in a round bottom flask. The solvents were removed using a rotary evaporator at a reduced pressure. The dried thin film was hydrated with PBS (pH 7.4) for 30 min at 10 °C above the phase transition temperature (T_c) of the amphiphiles while shaking. The dispersion was left for 4 h at room temperature (RT) to complete hydration and then stored at 4 °C overnight before use.

2.4. Determination of finasteride entrapment efficiency (EE%)

Finasteride-containing MLVs were separated from unentrapped drug by size exclusion chromatography (SEC) using Sephadex G-25 coarse gel and pH 7.4 PBS as eluting solvent. Empty liposomes/niosomes, extruded through 400 nm polycarbonate membrane (Nucleopore, Canada) were used to presaturate the column. Typically, a 100 μl sample of finasteride–nisomes was loaded on the column, then PBS was eluted at a flowing rate of 1 ml/min. The turbid fraction *containing vesicles* was collected and dissolved in methanol:PBS (1:1 v/v). The finasteride content of the vesicles was then determined by HPLC using C_{18} μBondapack Waters column (4.6 mm × 250 mm, 10 μm); the mobile phase consisted of 40:60 (v/v) acetonitrile:15 mM KH_2PO_4 (pH 4.5) and was pumped at a rate of 1.4 ml/min. Clobazam was used as internal standard. Finasteride entrapment efficiency is expressed as percent of initial amount of drug which was entrapped within

vesicles:

$$\text{EE\%} = \frac{\text{drug amount in turbid fraction}}{\text{total drug amount initially used}} \times 100$$

2.5. Vesicle characterization

The freshly prepared MLVs was first examined using light microscope (Leitz, Germany) and polarized light microscope (Olympus Optical Co., Japan) for the absence of any finasteride or cholesterol crystals or surfactant aggregates. The vesicle size distribution was determined by a single particle optical sensing (SPOS) method using Klotz® particle sizer (Germany). The average particle size was expressed as the area-number mean diameter (d_{AN}). The polydispersity, i.e. the width of the particle size distribution, was given by a SPAN index which was calculated by $(D_{0.9} - D_{0.1})/D_{0.5}$ where $D_{0.9}$, $D_{0.5}$ and $D_{0.1}$ are respectively the particle diameters determined at 90th, 50th and 10th percentile of particles undersized. SPAN was manually calculated from the data extracted from printouts of the particle sizer.

The transition temperature of niosomes and liposomes were determined by differential scanning calorimetry (Mettler TA 4000, DSC-30, Germany). The temperature and energy was calibrated by indium as standard. The liposomes and niosomes were prepared and concentrated by ultracentrifugation (Beckman, LS-50, 16,000 × *g*, for 30 min at 4 °C). Fifteen microliters of concentrated samples containing approximately 1.5 μmol lipid were placed in a small aluminium pan and sealed. An equal amount of PBS was placed in the reference pan. The samples were scanned at a rate of 5 °C/min, in the 0–80 °C range. Transition temperature was defined as trace moves off the baseline in the thermogram, i.e. the onset of the transition (T_c) (Ford and Timmins, 1989; Bouwstra et al., 1997).

2.6. Hydroalcoholic solution of finasteride

Finasteride and ^{3}H-finasteride were dissolved in ethanol: propylene glycol:PBS pH 7.4 (56:24:20 v/v) in order to obtain a final drug concentration of 0.53 mM (and 8 μCi/ml activity).

2.7. In vitro deposition and permeation studies

Full thickness of flank skin was excised from freshly sacrificed hamster with ether and its subcutaneous fat was carefully removed using scalpel and scissor. The skin was mounted on the vertical Franz diffusion cells (Ashk-e-shisheh, Tehran, Iran), with the epidermal side facing up (skin area of 0.502 cm^2, receiver volume of 8.5 ml). A minimum of three flank organs from three different animals was used for each formulation. The receiver chamber was filled with PBS (pH 7.4) solution and the temperature was maintained at 37 °C. Forty microliters of ^{3}H-finasteride liposomes or niosomes was applied on the skin and massaged for 2 min with a small glass spatula. All experiments were carried out under non-occluded conditions. Samples of 0.5 ml were withdrawn from the receiver chamber periodically and replaced with the same amount of PBS. The samples were mixed well with 5 ml Ready Value® liquid scintillation cocktail to produce a stable clear-phase gel.

After 24 h, the donor compartment was carefully rinsed with 300 μl buffer (5×). The skin patch was removed and fixed on a board using pins, with the epidermal side up. The flank skin was processed into its respective strata as follows. Eighteen pieces of adhesive tape, 1.9 cm wide and about 6 cm long, were used to complete stripping the stratum corneum (SC); three strips to remove the surface SC, seven strips to remove intermediate SC and eight strips for inner SC. The amount of drug remaining in the deeper skin strata was determined in the residual of the full thickness skin.

2.8. In vivo deposition experiments and scraping technique

Hamsters were anesthetized with ether followed by the intraperitoneal injection of ketamine HCl (50 mg/ml, 0.2 ml for the first injection, then 0.1 ml/h up to 10 h). For each formulation, three ears from three different animals were used. To ease application of formulation, hamsters were placed on their backs so that the ears lie flat (Lieb et al., 1994). Fifteen microliters of ^{3}H-finasteride liposomal or niosomal formulation was applied very slowly on the ventral mid section of ear with a micropipette. Care was taken here to prevent contamination of the edge or the dorsal side of the ear when applying formulations. The approximate area covered was about 0.4–0.5 cm^2. After 10 h, the hamsters were sacrificed in the ether-saturated tank and the ears were excised by cutting across the base and processed as follows. Each ear was mounted on a board with ventral side up; the surface was then gently padded with 4–6 cotton tubs to remove the formulation residue. Eighteen pieces of adhesive tape were used to remove the stratum corneum, as previously described. The tape was of sufficient size to cover the full area of the skin which was in contact with the formulation. A shiny and glossy appearance indicated that removal of the stratum corneum was complete.

Each ear remnant was further separated via scraping procedure, described by Niemiec et al. (1995) and Lieb et al. (1992, 1994), with some modifications. Each ear remnant was separated by gently peeling using a pair forceps such that the ventral side and the dorsal (non-treated) side of ear could be processed separately and the cartilage between them was associated with the dorsal ear. After peeling, the ventral ear was placed on a board with the epidermal side down using some pins. The sebaceous glands were removed by dragging a dull scalpel across the underside of the ventral dermis, under a stereoscopic microscope. The scraping was carried out with minimum force necessary to dislodge the pilosebaceous material having a milky appearance. The scraping procedure was performed three times (in different directions) to ensure complete removal of all the glands. This caused areas that were previously occupied by the sebaceous glands to appear as "hole" under a light microscope.

The tape strips were immersed in 5 ml Ready Protein+® scintillation cocktail for 72 h at 37 °C to dissolve the stratum corneum. To solubilize the tissue specimens, they were placed in hyamine hydroxide (tissue solubilizer, ICN) for 48–72 h at 37 °C. The solution was mixed with 1 ml hydrogen peroxide

(30%, v/v), which was added drop-wise, incubated for 24 h at RT, then mixed with Ready Value® scintillation cocktail containing 0.7% glacial acetic acid, and again incubated at RT for 2 days before counting. ^{3}H-finasteride was determined using a liquid scintillation counter (Beckman LS 6500 scintillation counter, USA). As a measure of targeting, the targeting ratio of finasteride was calculated using the following formula (Gupchup and Zata, 1997):

$$\text{targeting ratio} = \frac{\text{the amount of drug deposited in the pilosebaceousunits}}{\text{the total drug amount deposited in the skin}}$$

2.9. Data analysis and statistics

The data is expressed as mean ± S.D. Statistical analysis was performed by the Student's *t*-test or analysis of variance (ANOVA), followed by Tukey post hoc test using SPSS 11.5 for Windows. The level of significance was taken at *P*-values <0.05.

3. Results and discussion

3.1. Vesicle characteristics

The most stable, reproducible vesicular systems in our study were obtained when film hydration was performed at a temperature above the T_c of the amphiphiles existing in the formulations. Similar observations also have been reported by other workers (Lawrence et al., 1996). The entrapment efficiency of finasteride (EE%) in the recovered MLVs ranged from 80 to 97% (Table 1). The X-cross images observed under polarized light microscope indicated the lamellar structure of bilayer membrane (picture not shown). Brij76 formed discomes (large disk-shape vesicles) or MLVs where lower or higher cholesterol concentration was included. Brij72 formed polyhedral MLVs in a viscous gel medium (Fig. 1). Formation of discomes from non-ionic surfactants have been reported by other workers too (Uchegbu et al., 1996) and polyhedral MLVs (Uchegbu et al., 1997; Arunothayanun et al., 1999).

All of the vesicles formed were multilamellar with the mean area-number diameters (d_{AN}) ranging from 1.9 to 4.4 μm (Table 1). The size distribution profile is depicted in Fig. 2 and the SPAN index is shown in Table 1. A small SPAN indicates a narrow size distribution. Inclusion of the negatively charged lipid DCP in the formulation decreased the vesicle size, compared to un-charged niosomes, in most cases (data not shown). Smaller vesicles have been likely resulted from the increase in the curvature of the bilayer through the effect on electrostatic repulsion between the hydrophilic, ionised head groups (Uchegbu and Vyas, 1998).

In order to elucidate the influence of physical state of liposomes and niosomes on finasteride deposition in and permeation through hamster skin, amphiphiles of various characteristics were used to prepare vesicles. Since formation of niosomes from surfactants was achieved only where cholesterol was incorporated in the formulation, the DSC studies were carried out on niosomes, rather than surfactants alone. The DSC studies indicated that finasteride-containing vesicles comprising X:chol:DCP (see Table 1 for the description of formulations) were in the liquid state at the skin surface (i.e. having the $T_c < 32\,^{\circ}C$), where X was lecithin, DMPC, Brij97, or Brij76:Brij97 mixture (1:1, m.r.). Incorporation of Brij76, Brij72 and Span40 in the formulation (as X in the composition), however, produced gel-state vesicles with the transition temperatures of 35.4, 55.2, and 47 °C, respectively.

3.2. In vitro skin distribution and permeation

Mean percentage of ^{3}H-finasteride determined in isolated hamster flank skin following *in vitro* permeation studies are presented in Table 2. The total amount of finasteride penetrated into and permeated through hamster skin 24 h after topical application of liposomes and niosomes ranged from 5.5 to 13% of the initial dose, compared to 24% from hydroalcoholic solution ($P < 0.05$). The lower extent of drug permeation via MLVs may indicate that the lipid bilayers of niosomes/liposomes were rate limiting in drug permeation. Similarly, Bernard et al. (1995) suggested that liposomes clearly delayed the drug permeation compared with solution but better localized the drug in the sebaceous structure (targeting effect of liposome). Deposition of finasteride into the stratum corneum (strips 4–18) was significantly higher via hydroalcoholic solution compared to MLVs ($P < 0.05$). This

Table 1
Physical characteristics of finasteride-containing liposomes and niosomes prepared by film hydration method

Formulation composition	Vesicle size		Finasteride entrapment efficiency (%)
	d_{AN}[a] (μm)	Polydispersity (SPAN index[b])	
Lecithin:chol:DCP (8:2:1, m.r.)	1.97 ± 0.07	0.62 ± 0.05	82.0 ± 3
DMPC:chol:DCP (8:2:1, m.r.)	2.16 ± 0.13	1.56 ± 0.30	89.0 ± 7.6
Span40:chol:DCP (7:3:1, m.r.)	4.44 ± 0.15	2.35 ± 0.42	94.3 ± 4.9
Brij52:chol:DCP (7:3:1, m.r.)	3.66 ± 0.14	1.64 ± 0.33	96.3 ± 1.2
Brij72:chol:DCP (7:3:1, m.r.)	3.88 ± 0.23	3.32 ± 0.16	96.7 ± 1.5
Brij76:chol:DCP (7:3:1, m.r.)	2.95 ± 0.14	2.19 ± 0.16	79.7 ± 5.5
Brij97:chol:DCP (7:3:1, m.r.)	1.98 ± 0.06	0.61 ± 0.04	93.0 ± 3.5
Brij76:Brij97:chol:DCP (3.5:3.5:3:1, m.r.)	2.38 ± 0.08	0.85 ± 0.17	93.0 ± 1.0

Values represent mean ± S.D. ($n = 3$).
[a] Mean area-number diameter.
[b] Polydispersity/SPAN = [particle diameter at 90% cumulative size] − [particle diameter at 10% cumulative size]/[particle diameter at 50% cumulative size].

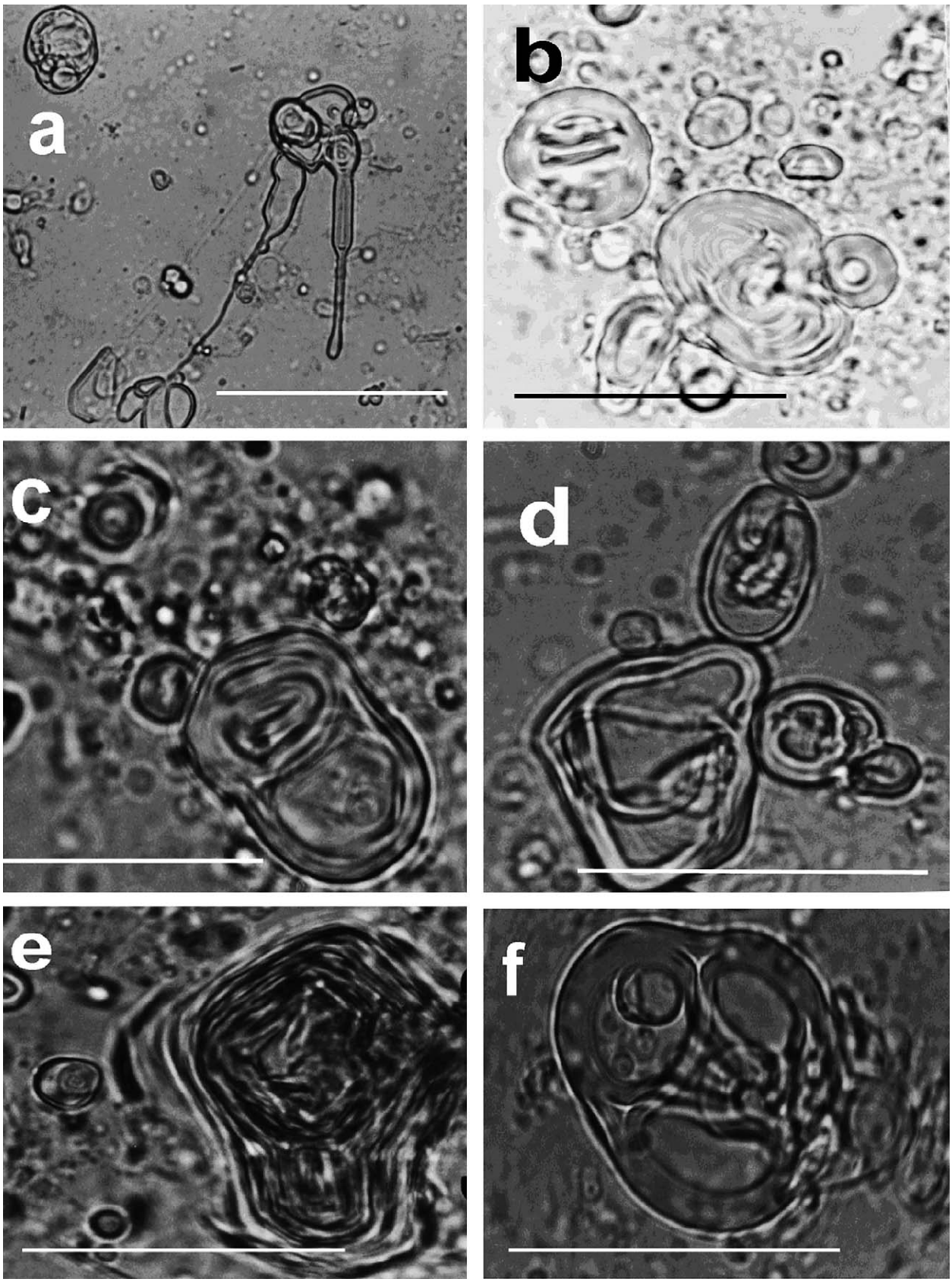

Fig. 1. Optical micrographs of finasteride-containing niosomes prepared by film hydration method. Pictures of vesicles of larger sizes are shown for their better resolution under light microscope. Liposomes and niosomes were composed of lecithin or DMPC:chol:DCP (8:2:1, m.r.) and surfactant:chol:DCP (7:3:1, m.r.), respectively. (a) Lecithin, (b) DMPC, (c) Span40, (d) Brij52, (e) Brij72 and (f) Brij97; "scale bar = 10 μm".

Table 2
The *in vitro* study of ^{3}H-finasteride deposition in and permeation through the hamster flank skin, 24 h following topical application of drug-containing negatively charged liposomal/niosomal formulations and hydroalcoholic drug solution (as control), using Franz diffusion cells

Formulation composition	Skin compartments (percent mean of applied dose ± S.D.)					Mass balance[a]
	Surface stratum corneum	Strips 4–10	Strips 11–18	Flank remain[b]	Percutaneous permeated[c]	
Ethanol:PG:PBS (56:20:24%, v/v)	72.3 ± 3.1	8.2 ± 0.8	5.1 ± 2.0	6.6 ± 0.8	4.1 ± 0.4	96.39 ± 4.77
Lecithin:chol:DCP (8:2:1, m.r.)	84.3 ± 8.3	1.5 ± 0.7	0.7 ± 0.1	1.7 ± 0.4	1.7 ± 0.3	89.9 ± 9.0
DMPC:chol:DCP (8:2:1, m.r.)	75.3 ± 8.0	2.3 ± 0.2	1.7 ± 0.6	7.8 ± 1.8	1.8 ± 0.47	88.9 ± 8.6
Span40:chol:DCP (7:3:1, m.r.)	81.0 ± 7.9	2.5 ± 0.8	1.1 ± 0.1	1.5 ± 0.4	1.4 ± 0.1	87.6 ± 8.6
Brij72:chol:DCP (7:3:1, m.r.)	82.9 ± 9.1	1.4 ± 0.4	1.1 ± 0.1	2.2 ± 0.6	1.8 ± 0.3	89.2 ± 8.0
Brij76:chol:DCP (7:3:1, m.r.)	81.5 ± 6.1	1.8 ± 0.5	1.1 ± 0.2	2.8 ± 0.9	2.5 ± 0.5	90.1 ± 6.6
Brij97:chol:DCP (7:3:1, m.r.)	84.9 ± 4.1	2.1 ± 0.2	1.5 ± 0.4	4.9 ± 0.6	2.6 ± 0.7	96.0 ± 3.4
Brij76:Brij97:chol:DCP (3.5:3.5:3:1, m.r.)	81.3 ± 10.4	2.9 ± 0.7	1.8 ± 0.3	4.3 ± 0.8	2.9 ± 0.7	92.5 ± 0.9

[a] Percent mean of applied dose determined in all skin compartments and the receiver chamber of Franz cell.
[b] Percent drug in the remaining of the flank.
[c] Determined in the receiver chamber of Franz diffusion cell.

could be due to quick fixation of finasteride to the stratum corneum following solvent evaporation. Moreover, ethanol is a very effective skin penetration enhancer and can extract stratum corneum lipids (Roberts and Cross, 2002). The amount of finasteride deposited within the different skin strata was lower from drug-containing gel-state Brij72 and Span40 niosomes and lecithin liposome, compared to liquid-state Brij97, Brij76:Brij97 and DMPC MLVs and also gel-state Brij76 niosomes, $P < 0.05$ (Table 2). An explanation is that non-ionic surfactants of higher hydrophilicity (i.e. Brij76 and Brij97) appear to increase leakage of drug from the vesicles (Uchegbu and Vyas, 1998), which as a result improves delivery of lipophilic molecules. The fraction of finasteride found in the dermis layer, following application of different formulations, was greatest where DMPC liposomes were used. Low transition temperature and penetration enhancing effect of DMPC may account for this high drug deposition. Similar findings by Ganesan et al. (1984) indicate that although liposomes clearly do not pass through the skin, they do induce remarkably different permeation behaviors of solute entrapped within them.

In order to administer finasteride via the topical route, a preparation is desirable which improves penetration into the skin, especially in sebaceous gland-containing zone, while reduces systemic absorption. MLV-mediated effect on finasteride permeation through flank skin is compared to hydroalcoholic solution in Fig. 3. The cumulative amount–time profiles of finasteride permeation followed the zero-order kinetics ($R^2 > 0.84$). The MLVs had a significantly greater retaining effect on finasteride

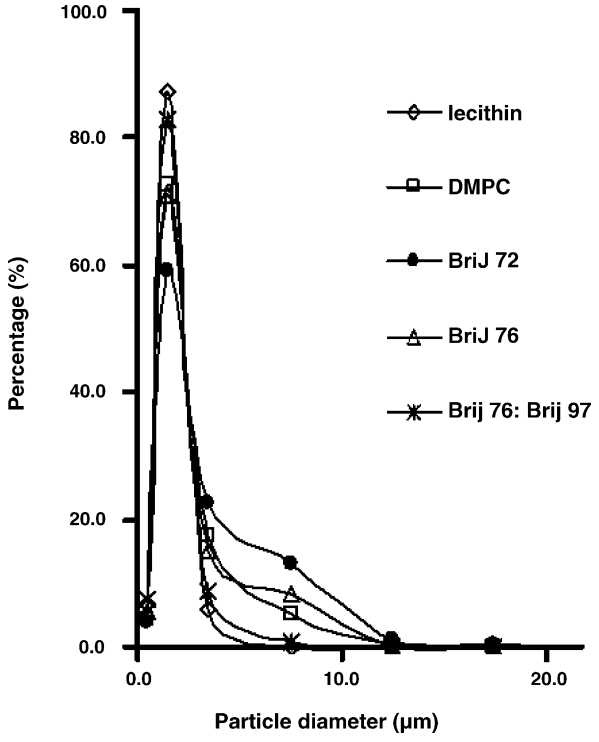

Fig. 2. The particle size distribution of finasteride-containing MLVs prepared by film hydration method. Liposomes and niosomes were composed of lecithin or DMPC:chol:DCP (8:2:1, m.r.) and surfactant or surfactants mixture:chol:DCP (7:3:1, m.r.), respectively.

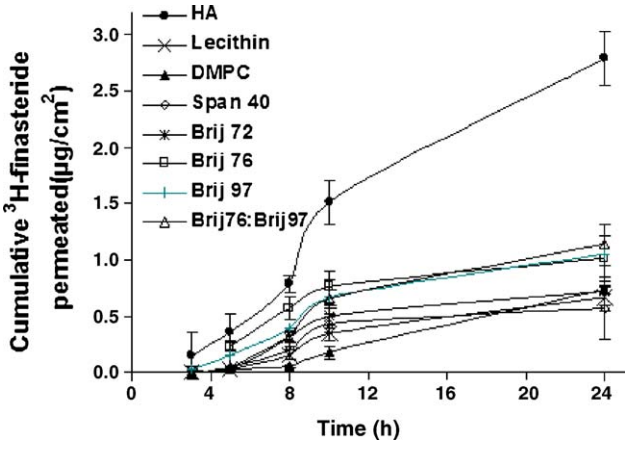

Fig. 3. Permeation profiles of ^{3}H-finasteride through hamster flank skin after topical application of drug-containing negatively charged niosomal/liposomal formulations and hydroalcoholic drug solution (HA) using Franz diffusion cells. Liposomes and niosomes were composed of lecithin or DMPC:chol:DCP (8:2:1, m.r.) and surfactant or surfactants mixture:chol:DCP (7:3:1, m.r.), respectively. Each point represents the mean ± S.D. ($n = 3$).

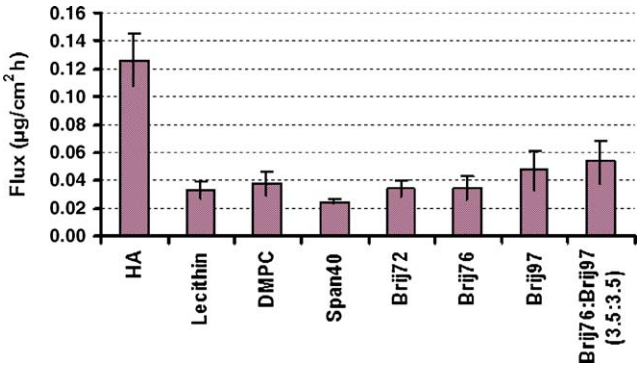

Fig. 4. The flux of ^{3}H-finasteride across hamster flank skin from various negatively charged niosomes/liposomes compared to hydroalcoholic drug solution (HA, as control). Liposomes and niosomes were composed of phospholipid (lecithin or DMPC):chol:DCP (8:2:1, m.r.) and surfactant (one or mixture of two):chol:DCP (7:3:1, m.r.), respectively. Each vertical bar and its error bar represent mean ± S.D. ($n = 3$).

compared to hydroalcoholic solution (ANOVA, $P < 0.01$). These results agree well with Ganesan et al. (1984) who reported that skin permeation of lipophilic active compounds was lower via liposomes than via solutions. The flux of finasteride from Brij76:Brij97 ($t = 3.3$, $P = 0.028$) and Brij97 ($t = 2.1$, $P < 0.01$) niosomes and DMPC liposome ($t = 2.2$, $P = 0.08$) was higher, where each compared with the flux of drug from gel-state Span40 niosome using Student's *t*-test. On the other hand, permeation rates of finasteride from gel-state Span40, Brij76, Brij72 niosomes and lecithin liposomes were not significantly different ($P > 0.05$) (Fig. 4). Differences in effect on drug transport between the liquid-state MLVs and the gel-state vesicles may be explained by differences in skin-formulation interactions, i.e. either due to the extent of interaction between vesicles and the stratum corneum or differences in partitioning of the drug between the vesicles and the stratum corneum (Ganesan et al., 1984). The surfactant molecules of liquid-state nature are thought to permeate into the intercellular lipid bilayers, thereby reduce the crystallinity of the intercellular lipid bilayers and thus increase the permeability of these bilayers (Sarpotdar and Zatz, 1986). In contrast, surfactant molecules forming rigid gel-state bilayers cannot penetrate into the stratum corneum, and thus they are not able to induce a penetration enhancing effect (Hofland et al., 1994). On the other hand, the packing nature of unsaturated fatty acids (i.e. Brij97) can change the fluidity of SC lipid structure and facilitate the skin permeation of drugs (Valjakka-Koskela et al., 1998; Fang et al., 2001) Thus, transfer of drug from the lipid bilayers into skin can occur as long as the bilayers are in a liquid-crystalline state.

3.3. In vivo deposition into the hamster ear compartments

The mean percentages of applied doses found in each of five isolated compartments of the hamster ear, following topical application of ^{3}H-finasteride MLVs and ^{3}H-finasteride hydroalcoholic solution are summarized in Table 3. Drug deposition in the PSU via vesicles was affected by composition, phase transition temperature of the amphiphiles and electrical charge. ^{3}H-finasteride vesicles comprising liquid-state amphiphiles, i.e.

Table 3
Mean percentage of ^{3}H-finasteride determined in isolated compartments of the ear at 10 h following *in vivo* application of finasteride-containing liposomes and niosomes and hydroalcoholic finasteride solution on the ventral surface of hamster ears, mean ± S.D. ($n = 3$)

Compartments	Hydroalcoholic	Compositions							
		Liposomes (lecithin or DMPC):chol:DCP (8:2:1, m.r.)		Niosomes (surfactant or surfactants mixture):chol:DCP (7:3:1, m.r.)					
		Lecithin	DMPC	Span40	Brij52	Brij72	Brij76	Brij97	Brij76:Brij97
Ear compartments									
Strips 4–10	8.43 ± 0.89	0.72 ± 0.19	1.66 ± 1.64	1.44 ± 0.33	2.36 ± 0.59	1.55 ± 0.62	0.53 ± 0.07	1.49 ± 0.49	1.93 ± 0.26
Strips 11–18	2.16 ± 0.84	0.61 ± 0.17	2.17 ± 0.69	0.62 ± 0.19	0.51 ± 0.09	0.55 ± 0.19	0.22 ± 0.09	0.61 ± 0.09	0.36 ± 0.10
Ventral (dermis)	1.06 ± 0.22	0.37 ± 0.14	0.80 ± 0.28	0.74 ± 0.20	0.41 ± 0.08	0.63 ± 0.18	0.26 ± 0.01	0.44 ± 0.14	0.82 ± 0.07
Pilosebaceous unit	0.76 ± 0.28	0.41 ± 0.14	2.05 ± 0.69	0.51 ± 0.12	0.76 ± 0.09	0.41 ± 0.14	0.35 ± 0.10	1.32 ± 0.46	2.33 ± 0.56
Dorsal/cartilage	0.97 ± 0.11	0.29 ± 0.09	0.99 ± 0.34	0.54 ± 0.08	1.67 ± 0.66	0.72 ± 0.09	0.52 ± 0.15	0.86 ± 0.34	0.68 ± 0.06
Sum (strips 4 to dorsal)	13.37 ± 0.84	2.41 ± 0.4	7.67 ± 2.80	3.85 ± 0.63	5.71 ± 0.42	3.86 ± 0.91	1.88 ± 0.33	4.73 ± 0.33	6.12 ± 0.83
Targeting ratio[a]	0.06 ± 0.01	0.17 ± 0.03	0.27 ± 0.04	0.13 ± 0.03	0.13 ± 0.03	0.11 ± 0.04	0.19 ± 0.05	0.18 ± 0.06	0.38 ± 0.04

[a] Targeting ratio refers to the amount of finasteride deposited in the pilosebaceous unit divided by the total amount deposited in the skin (strips 4 to dorsal).

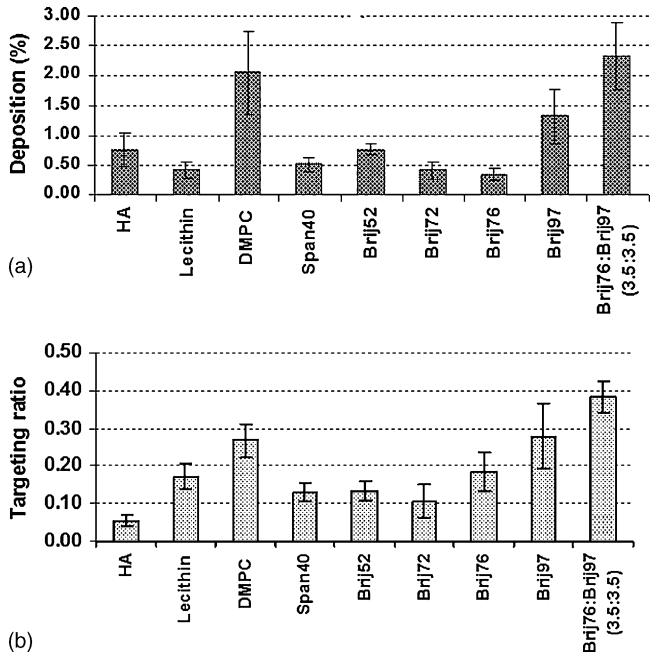

Fig. 5. ^{3}H-finasteride deposition (a) into and its targeting ratio (b) in the pilosebaceous units of hamster ear mediated by negatively charged niosomes/liposomes and a hydroalcoholic drug solution (HA) (data represents mean ± S.D., $n = 3$).

Brij97, Brij76:Brij97 mixture, or DMPC and bearing negative charge (due to having dicetyl phosphate in their composition) produced high drug deposition in the PSU (Fig. 5a and b) and dorsal skin (Table 3) but low drug deposition in the stratum corneum and dermis (Table 3). The topically applied formulation undergoes dehydration at the temperature of the skin, i.e. ~32 °C. At this temperature, as determined from differential scanning calorimetric thermograms, niosomes made of Brij52, Brij76, Brij72 and Span40 remain in the gel-state, whereas vesicles comprising amphiphiles of liquid-state nature, i.e. having the $T_c < 32$ °C, begin to melt, resulting in the bilayer fluidization, and partial release of the amphiphiles from the bilayers. The difference in drug deposition ($P < 0.05$) between Brij76 niosome and Brij76:Brij97 niosomes (Table 3, Fig. 5a and b), thus can be partially attributed to the physical state of the vesicles. The release of molecules of polyoxyethylene-10 alkyl ethers (i.e. Brij76 and Brij97), which are known to have permeation enhancing effect (Niemiec et al., 1995), can also attribute to greater deposition into the PSU. Findings by Weiner and coworkers (Lieb et al., 1994; Niemiec et al., 1995) also indicated that deposition of drugs to the PSU were enhanced by incorporation of glyceryl dilaurate, a lipid which fluidizes the bilayers, into the polyoxyethylene-10 stearyl ether niosome formulations.

Although hydroalcoholic solution of finasteride, compared to all other formulations, produced the highest drug deposition in the stratum corneum (strips 4–18), it had a targeting ratio of 0.06 in the pilosebaceous unit, which was much lower than 0.38 obtained from Brij76:Brij97 niosomes (Fig. 5b). Similar findings by several groups (Egbaria and Weiner, 1990; Lieb et al., 1992; Fleisher et al., 1995; Niemiec et al., 1995; Bernard et al., 1997) supports the superiority of niosomes and liposomes to hydroalcoholic solutions or other conventional formulations in enhancing targeting drug to the PSU. Alcoholic vehicles are lipid solvents that can increase the fluidity of lipids within the stratum corneum. They also act on the sebum within the follicles and cause rapid migration of a solute into the PSU, thereby making the transfollicular pathway predominant in the initial stages of absorption, followed by the drug deposition in all skin strata (Roberts and Cross, 2002).

An examination of the data in Table 3 reveals in general that the amounts of drug found in the cartilage/dorsal ear are proportional to the amount in the pilosebaceous unit. Liquid-state vesicles composed of Brij97 or DMPC produced higher dorsal as well as pilosebaceous deposition compared to gel-state niosomes and egg lecithin liposomes. Niemiec et al. (1995) proposed that increased deposition into the cartilage/dorsal ear may have been resulted from the clearance of the drug by the vast vascular network from the vicinity of the glands.

The nearly two- to six-fold enhancements of finasteride deposition in the pilosebaceous unit by liquid-state MLVs compared to gel-state niosomes might support the hypothesis of dual action for these multilamellar vesicles; deposition near the follicular ducts that contain lipids and more enhancing affect on the permeation with fluid configuration of vesicles. However, freeing up of part of non-ionic lipids results reduction of the barrier properties of stratum corneum (Schreier and Bouwstra, 1994; Manosroi et al., 2003) and contributes to permeation enhancement by vesicles.

Although it is generally believed that intact liposomes do not penetrate into the compact layers of the stratum corneum (Weiner et al., 1989), the specific mechanisms of liposome action in the skin strata remain to be enlightened. Intact liquid-crystalline state MLVs may also enter and traverse the hair follicle (Touitou et al., 1994; Gupchup and Zata, 1997), or develop into a structural film which seemingly fills the follicular opening, intimately mixes with the follicular contents, and fosters drug diffusion to the depths of the gland (Touitou et al., 1994; Lieb et al., 1994; Lauer et al., 1996; Gupchup and Zata, 1997). Lauer et al. (1995) proposed a theory that upon dehydration, the liposomal bilayers may yield a fluid liquid-crystalline state in which bilayers containing drug can partition and pack into the follicular ducts that contain lipids. On the other hand, vesicle disintegration in the sebum-filled hair follicle is possible (Lieb et al., 1992); that may affect liposomal integrity in a way that abolishes the distinction between entrapped or free drug associated with liposomal bilayers. Although it is generally believed that the intercellular route may dominate during steady state penetration of compounds, it has been argued that the skin appendages (hair follicles, pilosebaceous and eccrine glands) may offer an alternative pathway for a diffusing molecule (Roberts and Cross, 2002).

4. Conclusion

The MLVs had a significantly greater retaining effect on finasteride permeation compared to hydroalcoholic solution (ANOVA, $P < 0.01$). Negatively charged DMPC, Brij97 MLVs and Brij76:Brij97 MLVs, all being in the liquid-state, facili-

tated the deposition of finasteride into sebaceous gland region and reduced its percutaneous absorption, where compared with hydroalcoholic solution. In contrast, vesicles composed of surfactants of gel-state nature did not enhance delivery of the drug to the pilosebaceous units, possibly due to lower interaction with stratum corneum and lack of penetration into its dense layers. Therefore, the phase transition temperature of the amphiphile used to prepare vesicles from may play an important role in permeation of drug through skin. Better understanding of the control mechanisms, which govern the complex structure of the pilosebaceous unit, will help designing more rationally the hair follicle-specific drug delivery systems. However, in order to truly ascertain the significance of follicular delivery, appropriate models and quantitative methods must still be developed.

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