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Deformable liposomes and ethosomes: Mechanism of enhanced skin delivery

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Abstract

Despite intensive research, the mechanisms by which vesicular systems deliver drugs into intact skin are not yet fully understood. In the current study, possible mechanisms by which deformable liposomes and ethosomes improve skin delivery of ketotifen under non-occlusive conditions were investigated. In vitro permeation and skin deposition behavior of deformable liposomes and ethosomes, having ketotifen both inside and outside the vesicles (no separation of free ketotifen), having ketotifen only inside the vesicles (free ketotifen separated) and having ketotifen only outside the vesicles (ketotifen solution added to empty vesicles), was studied using rabbit pinna skin. Results suggested that both the penetration enhancing effect and the intact vesicle permeation into the stratum corneum might play a role in improving skin delivery of drugs by deformable liposomes, under non-occlusive conditions, and that the penetration enhancing effect was of greater importance in case of ketotifen. Regarding ethosomes, results indicated that ketotifen should be incorporated in ethosomal vesicles for optimum skin delivery. Ethosomes were not able to improve skin delivery of non-entrapped ketotifen.

Keywords: Deformable liposomes; Ethosomes; Skin delivery; Transdermal; Topical; Mechanism; Ketotifen fumarate

1. Introduction

For the last decades, topical delivery of drugs by liposomal formulations has evoked a considerable interest. Despite intensive research, results of the interaction of liposomes with skin are contradictory (Kirjavainen et al., 1999a). Recently, it became evident that traditional liposomes are of little or no value as carriers for transdermal drug delivery, because they do not deeply penetrate skin, but rather remain confined to upper layers of the stratum corneum (Touitou et al., 2000). Confocal microscopy studies showed that intact liposomes were not able to penetrate into the granular layers of the epidermis (Kirjavainen et al., 1996). The possible mechanisms by which traditional liposomes could improve skin delivery of drugs have been extensively studied and reviewed (Schreier and Bouwstra, 1994; Kirjavainen et al., 1996, 1999b; Bouwstra and Honeywell-Nguyen, 2002; Williams, 2003; El Maghraby et al., 2006).

Recent approaches in modulating drug delivery through skin have resulted in the design of two novel vesicular carriers, deformable liposomes and ethosomes. Deformable liposomes (Transfersomes®) are the first generation of elastic vesicles introduced by Cevc et al. and were reported to penetrate intact skin carrying therapeutic concentrations of drugs, but only when applied under non-occluded conditions (Cevc and Blume, 1992). They consist of phospholipids and an edge activator. An edge activator is often a single chain surfactant that destabilizes lipid bilayers of the vesicles and increases deformability of the bilayers (Honeywell-Nguyen and Bouwstra, 2005). Sodium cholate, Span 80, Tween 80 and dipotassium glycyrrhizinate were employed as edge activators (Cevc, 1996; El Maghraby et al., 1999, 2000a,b; Trotta et al., 2004). Several studies have reported that deformable liposomes were able to improve in vitro skin delivery of various drugs (El Maghraby et al., 1999, 2001; Trotta et al., 2002, 2004; Boinpally et al., 2003) and to penetrate intact skin in vivo, transferring therapeutic amounts of drugs (Cevc and Blume, 2001, 2003, 2004), with efficiency comparable with subcutaneous administration (Cevc et al., 1995, 1998; Paul et al., 1995; Cevc, 2003). In a recent study, the provesicular approach, proposed to enhance the stability of vesicles, has been extended to deformable liposomes and proultraflexible lipid vesicles of levonorgestrel were also developed and investigated (Jain et al., 2005). van den Bergh (1999) introduced a second generation of elastic vesicles consisting mainly of non-ionic surfactants. These surfactant-based elastic vesicles were

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shown to be more effective than rigid vesicles in enhancing skin penetration of various chemical entities (Honeywell-Nguyen et al., 2002, 2003a,b; Honeywell-Nguyen and Bouwstra, 2003). Ethosome is another novel lipid carrier, recently developed by Touitou et al., showing enhanced skin delivery. The ethosomal system is composed of phospholipid, ethanol and water (Touitou et al., 2000). Ethosomes were reported to improve skin delivery of various drugs (Dayan and Touitou, 2000; Touitou et al., 2000; Ainbinder and Touitou, 2005; Paolino et al., 2005). The mechanisms by which vesicular systems deliver drugs into intact skin are not yet fully understood.

In a previous study (Elsayed et al., 2006), traditional liposomes, deformable liposomes and ethosomes were investigated as carriers for skin delivery of a model drug, ketotifen fumarate (KT). Traditional liposomes improved only skin deposition of KT. These results were in accordance with most recent publications concerning traditional liposomes, showing that traditional liposomes are of little or no value as carriers for transdermal drug delivery because they do not deeply penetrate skin, but rather remain confined to upper layers of the stratum corneum showing only a localizing effect with more drug in skin strata. Both deformable liposomes and ethosomes improved skin delivery (permeation and deposition) of KT with greater improvement of KT skin deposition than improvement of KT skin permeation.

The aim of the present study was to investigate possible mechanisms by which deformable liposomes and ethosomes could improve skin delivery of the model hydrophilic drug, ketotifen fumarate (KT), under non-occlusive conditions. KT is also a good candidate for a transdermal delivery system (Nakamura et al., 1996; Chiang et al., 1998). Several studies were carried out to examine and enhance in vitro transdermal absorption of KT and to develop suitable transdermal delivery systems for the drug (Lee et al., 1994; Nakamura et al., 1996; Kobayashi et al., 1997; Chiang et al., 1998; Inoue et al., 2000; Kitagawa and Ikarashi, 2003). In the present study, we investigated in vitro permeation and skin deposition behavior of deformable liposomes (DL) (prepared using Tween 80 as an edge activator) and ethosomes (ES), having KT both inside and outside the vesicles (no separation of non-entrapped KT), having KT only inside the vesicles (free KT separated by ultracentrifugation) and having KT only outside the vesicles (empty vesicles to which KT solution was added). This design is similar to that described previously by Verma et al. (2003a).

2. Materials and methods

2.1. Materials

Lipoid S 100 (Phosphatidylcholine (PC) from soybean lecithin), containing not less than 94% PC (95.8% in the batch used in present study), was a kind gift from Lipoid GmbH (Ludwigshafen, Germany). Polyoxyethylene sorbitan monooleate (Tween 80) was from ADWIC, El-Nasr Pharmaceutical Chemicals Co. (Abu Zaabal, Egypt). Ketotifen fumarate (KT) was kindly supplied by Laboratori Alchemia (Milano, Italy). All other chemicals were of analytical grade and used as received.

2.2. Preparation of deformable liposomes

Deformable liposomes with KT both inside and outside the vesicles (DL-In/Out) were prepared by the conventional mechanical dispersion method. Briefly, Lipoid S 100 (for final PC concentration of 4.25% (w/v)) and the edge activator (Tween 80 in a PC-Tween 80 ratio of 84.5:15.5 (w/w)) were dissolved in ethanol. Drug was added to furnish the desired concentration in the final preparation (0.5%, w/v). Organic solvent was removed by rotary evaporation (Rotavapor, Buchi, Germany) above the lipid transition temperature (43 °C). Final traces of solvent were removed under vacuum, overnight. Deposited lipid film was hydrated with 7% (v/v) ethanol in distilled water by rotation at 100 rpm for 30 min at the corresponding temperature. Resulting vesicles were allowed to swell for 2 h at room temperature. Liposomal suspensions were then sonicated for 20 min.

For the preparation of deformable liposomes with KT only inside the vesicles (DL-In), free KT was removed by ultracentrifugation (3K 30 refrigerated centrifuge, Sigma Laborzentrifugen GmbH, Germany), for two cycles, at 23 000 rpm, at 5 °C, for 1 h each. Purified sediment was then diluted to the initial volume using 7% (v/v) ethanol in distilled water, in order to maintain a final PC concentration of 4.25% (w/v), and used immediately for in vitro permeation and skin deposition study. KT concentration was analyzed by HPLC after vesicle lysis using methanol.

For the preparation of deformable liposomes with KT only outside the vesicles (DL-Out), blank deformable liposomes were first prepared with higher PC and Tween 80 concentrations (in the same ratio). Just prior to application, a pre-calculated volume of suitable KT solution in 7% (v/v) ethanol in distilled water was used for dilution of the blank formulation to get a final liposomal suspension containing the same final PC, Tween 80 and KT concentrations.

2.3. Preparation of ethosomes

Ethosomes with KT both inside and outside the vesicles (ES-In/Out) were prepared as described previously (Dayan and Touitou, 2000; Touitou et al., 2000). Briefly, Lipoid S 100 (for final PC concentration of 4.25% (w/v) in 30% (v/v) ethanol) and the drug (for final KT concentration of 0.5% (w/v)) were dissolved in ethanol. Distilled water was added slowly in a fine stream at constant rate in a well-sealed container with constant mixing by a magnetic stirrer at 700 rpm. Mixing was continued for additional 5 min. The system was kept at 30 °C throughout the preparation. Similar procedures were carried out to prepare ethosomes with KT only outside the vesicles (ES-Out) and with KT only inside the vesicles (ES-In) as those described for deformable liposomes.

2.4. In vitro permeation and skin deposition studies

Rabbit pinna skin from 1.5 to 2 kg male albino rabbits (University of Alexandria, Egypt) was used. Pinna skin, including epidermis and dermis, was taken from the inner side of the ear, after sacrificing the animals, by cutting along the tip of the ear and peeling the skin from the underlying cartilage (Corbo et al.,

1990; Touitou et al., 2000). A preliminary wash of the skin was done with normal saline, followed by drying between two filter papers. Skin was used directly without storage. All animals were treated in accordance with the institutional laboratory animal care approved ethical guidelines.

Experiments were run in Franz diffusion cells having a receptor compartment volume of 8 ml. The in vitro study design used in the present study was similar to that described by El Maghraby et al. (1999, 2000a). Experiments were performed in two stages. The first stage was used in determination of the drug permeating the skin. This stage used pH 7.4 isotonic phosphate buffer containing 0.11% (w/v) formaldehyde as preservative (Sloan et al., 1991; Beall and Sloan, 2001; Kanikkannan et al., 2001), as the receptor medium. Skin membranes were mounted, with the stratum corneum side up and the donor compartment dry and open to atmosphere, and floated on receiver solution for 24 h for equilibration and pre-hydration (open hydration protocol) (El Maghraby et al., 1999), in order to maintain a transepidermal hydration gradient (Warner et al., 1988) which has been proposed as generating the driving force for skin penetration of deformable liposomes (Cevc and Blume, 1992). The receiver content was then replaced by a fresh medium. Test formulations (150 μl non-occluded open application) were applied to skin surface, which had an available diffusion area of 3.14 cm^2, and left to dry. Aqueous KT (0.5%, w/v) solution and KT solution (0.5%, w/v) in 30% (v/v) ethanol in distilled water were used as controls ($n = 3$). Three-milliliter samples of the receptor were removed at appropriate intervals for HPLC assay and immediately replaced with fresh medium.

At the end of this stage (24 h), the donor compartment and the skin surface were washed five times with warm (45 °C) receptor medium. The second stage was employed to determine skin deposited drug. The receptor content was completely removed and replaced by 50% (v/v) ethanol in distilled water (El Maghraby et al., 1999) and kept for a further 12 h followed by HPLC assay. This receiver solution (50% (v/v) ethanol in distilled water) was suggested to diffuse through skin, disrupting any liposome structure and extracting deposited drug from skin, thus giving a measure of skin deposition (El Maghraby et al., 1999, 2000a, 2001).

The receptor medium was kept at 37 ± 1 °C throughout experiments, in order to maintain the skin surface at 32 °C. Each vesicular system was investigated in five cells.

2.5. HPLC assay

The concentration of KT was determined by HPLC. HPLC method used was a previously developed method (Elsayed, 2006) with slight modifications. HPLC System was Perkin-Elmer Series 200 equipped with Series 200 LC pump, Series 200 autosampler, Series 200 UV/Vis detector, Series 600 interface and TotalChrom Navigator 6.2.0.0 Computerized Chromatography Analysis Software. Separation was carried out on Spheri-5, RP-8 column (Perkin-Elmer) with a particle size of 5 μm. The mobile phase was a mixture of methanol and triethylamine acetate buffer (pH 3.5; 0.035 M) (70:30, v/v) at a flow rate of 1.5 ml/min. UV detection was performed at a wavelength of 297 nm. The method was validated for selectivity, linearity, accuracy and precision.

2.6. Statistical analysis

All reported data are mean ± S.E.M. Statistical significance was checked by Student's *t*-test and considered to be granted at $P < 0.05$, unless otherwise indicated.

3. Results

Deformable liposomes and ethosomes prepared in the current study were found to have mean vesicle size of 108.6 ± 6.4 and 91.2 ± 3.7 nm (CILAS 1064, Cilas, USA) and KT entrapment efficiency of 74.51 ± 0.86 and 43.98 ± 0.96% (ultracentrifugation method (Heeremans et al., 1995)), respectively.

Table 1 shows cumulative KT permeated and skin deposited after 24 h from controls. Data are expressed as % of total KT in the applied dose. KT solution in 30% ethanol showed a reduction in cumulative KT permeated and skin deposited after 24 h relative to aqueous control; however, differences were not significant ($P > 0.05$).

Fig. 1A shows in vitro skin permeation profiles of KT from deformable liposomes. Cumulative KT that permeated the skin after 24 h of non-occlusive application was significantly higher for DL-Out (8.40 ± 0.77%, $P < 0.001$) than for DL-In (3.22 ± 0.29%). DL-Out also improved slightly, but not significantly ($P > 0.05$), cumulative KT that permeated the skin after 24 h over DL-In/Out (6.59 ± 0.43%). DL-Out showed an initial lower flux, with 4 h lag time, than DL-In/Out and DL-In, that was followed by gradual increase in flux. Regarding skin deposition, the quantity of skin deposited KT after 24 h of non-occlusive application was also significantly higher from DL-Out (45.96 ± 3.69%) than from DL-In/Out (33.05 ± 2.31%, $P < 0.05$) and from DL-In (20.49 ± 4.41%, $P < 0.01$) (Fig. 1B). Only DL-Out and DL-In/Out showed significant improvement in cumulative KT permeated and skin deposited after 24 h over aqueous control. DL-In only improved slightly and not significantly ($P > 0.05$) skin deposited KT over aqueous control.

Fig. 2A shows in vitro skin permeation profiles of KT from ethosomes. Cumulative KT that permeated the skin after 24 h of non-occlusive application was significantly higher for ES-In (11.12 ± 1.89%, $P < 0.01$) than for ES-Out (2.07 ± 0.49%). ES-In also improved cumulative KT that permeated the skin after 24 h over ES-In/Out (4.77 ± 0.15%). Regarding skin deposition, the quantity of skin deposited KT after 24 h of non-occlusive application, surprisingly, was significantly higher from ES-In/Out (49.36 ± 1.81%) than from ES-In

Table 1
Cumulative ketotifen permeated and skin deposited after 24 h from controls

	Drug permeated (%)	Drug deposited in skin (%)
KT solution in 30% ethanol	3.36 ± 0.13	7.97 ± 1.66
Aqueous KT solution	4.05 ± 0.22	15.11 ± 3.04

Data are expressed as % of total KT in the applied dose (mean ± S.E.M., $n = 3$).

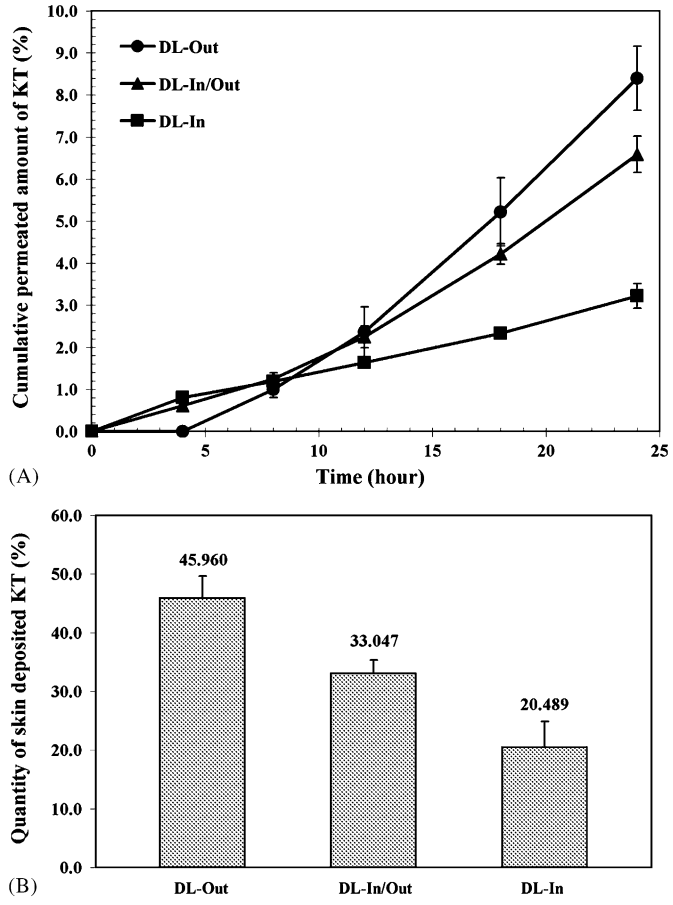

Fig. 1. (A) Cumulative KT that permeated the skin in vitro from deformable liposomes over time. (B) Skin deposited KT in vitro after 24 h of application of deformable liposomes. Data are expressed as % of total KT in the applied dose (mean ± S.E.M., $n = 5$).

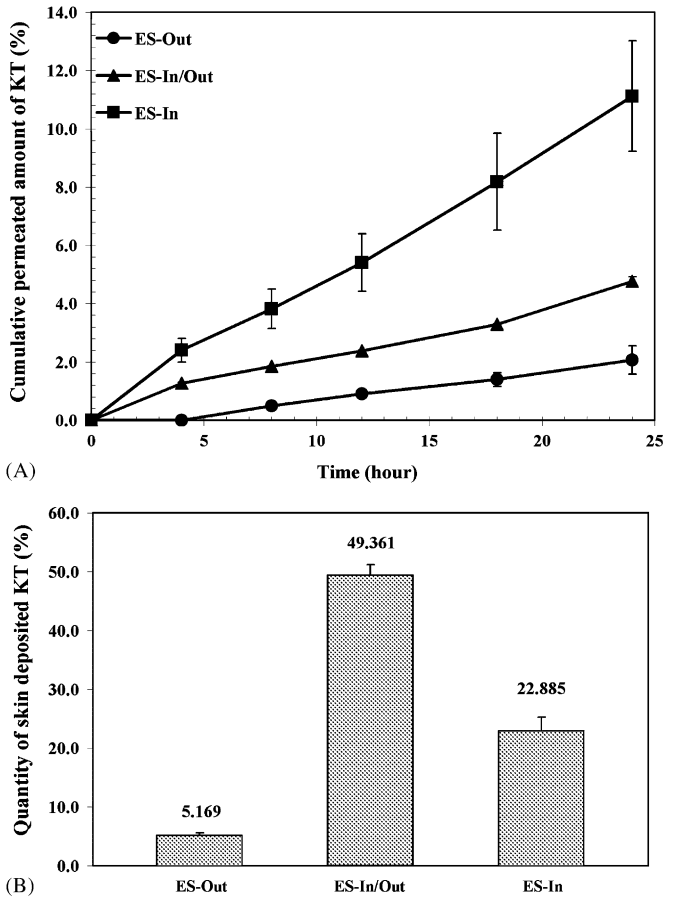

Fig. 2. (A) Cumulative KT that permeated the skin in vitro from ethosomes over time. (B) Skin deposited KT in vitro after 24 h of application of ethosomes. Data are expressed as % of total KT in the applied dose (mean ± S.E.M., $n = 5$).

(22.89 ± 2.40%, $P < 0.001$) and from ES-Out (5.17 ± 0.47%, $P < 0.0001$) (Fig. 2B). Only ES-In and ES-In/Out showed significant ($P < 0.01$) improvement in cumulative KT permeated and skin deposited after 24 h over KT solution in 30% ethanol. However, ES-Out showed slight, but not significant ($P > 0.05$) reduction in cumulative KT permeated and skin deposited after 24 h relative to KT solution in 30% ethanol.

4. Discussion

In the present study, we investigated the in vitro permeation and skin deposition behavior of both deformable liposomes (DL), prepared using Tween 80 as an edge activator, and ethosomes (ES). Tween 80 was selected based on previous study findings (Elsayed et al., 2006) in which KT deformable liposomes with Tween 80 as an edge activator showed higher KT entrapment efficiency and were more stable upon storage at 5 ± 1 °C than KT deformable liposomes prepared using sodium cholate or sodium deoxycholate.

Several studies investigated possible mechanisms by which deformable vesicles could improve skin delivery of drugs. Two mechanisms were proposed (Honeywell-Nguyen et al., 2003a; Honeywell-Nguyen and Bouwstra, 2003). First, vesicles can act as drug carrier systems, whereby intact vesicles enter the stratum corneum carrying vesicle-bound drug molecules into the skin (mechanism 1). Second, vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and subsequently modify the intercellular lipid lamellae. This will facilitate penetration of free drug molecules into and across the stratum corneum (mechanism 2).

The first mechanism was put forward by Cevc et al. for deformable liposomes (Cevc and Blume, 1992, 2001; Cevc et al., 2002). It was also claimed that intact deformable liposomes penetrated through the stratum corneum, under the influence of the naturally occurring in vivo tanscutaneous hydration gradient (Cevc and Blume, 2001) and through the underlying viable skin into blood circulation (Cevc et al., 2002). The latter claim was met with much skepticism (Bouwstra et al., 2003). Several studies supported that surfactant-based elastic vesicles and deformable liposomes may act as carrier systems (El Maghraby et al., 2000b; Honeywell-Nguyen et al., 2003a; Honeywell-Nguyen and Bouwstra, 2003; Verma et al., 2003b).

Recently, an in vivo electron microscopic study demonstrated a fast (within 1 h of application) partitioning of intact surfactant-based elastic vesicles into human stratum corneum, but almost no vesicles could be found in the deepest layers of the stratum corneum (Honeywell-Nguyen et al., 2000). Recent evidence

showed that the water gradient across the skin may not be linear and there may be a relatively 'dry' region within the stratum corneum (Williams, 2003). It was also noticed that even in fully hydrated state, the water content in the lowest stratum corneum layers close to the viable epidermis is much lower than in central regions of the stratum corneum. Therefore, it was expected that, as a result of the osmotic force, vesicles will not penetrate beyond the level of the lowest layers in the stratum corneum (Bouwstra and Honeywell-Nguyen, 2002; Honeywell-Nguyen and Bouwstra, 2003). Results of studies showing that deformable liposomes only improved skin deposition of some drugs (El Maghraby et al., 2001; Trotta et al., 2002) could support this proposal. Drugs will penetrate further solitary. Thus, drugs have to be released from vesicles in order to reach the systemic circulation (Honeywell-Nguyen and Bouwstra, 2003).

Several studies suggested also a possible penetration enhancing mechanism (mechanism 2) for surfactant-based elastic vesicles and for deformable liposomes (van den Bergh et al., 1999; El Maghraby et al., 2001; Verma et al., 2003a). To the best of our knowledge, all these studies involved hydrophilic drugs or hydrophilic fluorescent compounds. Deformable liposomes also improved only skin deposition of dipotassium glycyrrhizinate (Trotta et al., 2002) and 5-fluorouracil (El Maghraby et al., 2001), hence are only useful for dermal delivery of these hydrophilic drugs. The limited partitioning into the acceptor phase in these studies indicates that deformable vesicles are not carrying associated drug into the acceptor phase.

El Maghraby et al. (2006) suggested that the reasons for variable effects and explanations may arise from different vesicle compositions, alternative methods of preparation which result in vesicles having diverse characteristics with respect to size, lamellarity, charge, membrane fluidity and elasticity and drug entrapment efficiency, and the selection of skin membranes (man or animal, in vivo or in vitro). Other aspects of the experimental design (such as receptor solution composition) and the technique used in evaluation may have profound effects on the recorded action.

In the current study, DL-Out significantly improved cumulative KT permeated and skin deposited after 24 h of non-occlusive application over DL-In. Differences in results might have been reduced by possible water and drug exchange across vesicular bilayers (Cevc et al., 2003); however, differences were sufficiently statistically significant ($P < 0.01$). DL-Out also significantly improved skin deposited KT after 24 h of non-occlusive application over DL-In/Out, however, slight ($P > 0.05$) improvement in cumulative KT that permeated the skin after 24 h of non-occlusive application over DL-In/Out was only observed. Only DL-Out and DL-In/Out showed significant improvement in cumulative KT permeated and skin deposited after 24 h over aqueous control. DL-In improved only slightly and non-significantly ($P > 0.05$) skin deposited KT over aqueous control and failed to show any appreciable improvement in skin permeation.

The current study findings suggest that the penetration enhancing effect might be of greater importance, in the enhanced skin delivery of KT by deformable liposomes under non-occlusive conditions, than intact vesicle permeation into the stratum corneum. Possible interaction of vesicles with layers of the stratum corneum, promoting impaired barrier function of these layers to the drug, with less well-packed intercellular lipid structure forms (Essa et al., 2003), and with subsequent increased skin partitioning of the drug (Kirjavainen et al., 1999a) could be supported. We therefore support that both the penetration enhancing effect and the intact vesicle permeation into the stratum corneum play a role in the enhanced skin delivery of drugs by deformable liposomes under non-occlusive conditions and suggest that one of the two mechanisms might predominate according to the physico-chemical properties of the drug.

The transport of the drug carried by deformable liposomes into the stratum corneum bypassing the main barrier for drug permeation will considerably improve skin delivery. This role may be of great effect in improving skin deposition. However, several factors might contribute to or contribute against this role in improving transdermal flux. Drug release from the vesicles in the stratum corneum is an important step (Honeywell-Nguyen and Bouwstra, 2003) that will affect transdermal flux. The rate and amount of released drug is a balance between two factors: (1) drug affinity to vesicles, and (2) drug solubility in lipids of the stratum corneum (Honeywell-Nguyen and Bouwstra, 2003). As reported in a recent study, poor drug release resulted in retention of the drug within vesicles in the stratum corneum and elastic vesicles served as a slow release depot system (Honeywell-Nguyen and Bouwstra, 2003).

For hydrophilic drugs, the penetration enhancing effect seems to play a more important role in the enhanced skin delivery than in case of lipophilic drugs (as for many penetration enhancers), since permeation of hydrophilic molecules tends to be relatively slower and hence more enhanceable (Williams and Barry, 1991; Williams, 2003). Results of the current study and other studies (El Maghraby et al., 2001; Verma et al., 2003a) support the existence of an important penetration enhancing effect. The intact vesicle permeation mechanism will have also an important role specially in improving skin deposition. However, as previously mentioned, drug release from vesicles in the stratum corneum is an important step that affects transdermal flux (Honeywell-Nguyen and Bouwstra, 2003). We therefore suggest that, regarding transdermal permeation, hydrophilic drugs might not be necessarily entrapped in vesicles for optimum effects. On the contrary, entrapment of hydrophilic drugs might result in a slow release, where phospholipids might form an extra lipid barrier, decreasing the flux of these hydrophilic drugs. This could explain the current study findings where entrapment of KT failed to improve its transdermal permeation. This also could explain the variation in reported results regarding improvement of skin permeation of hydrophilic drugs by deformable liposomes, as some studies showed improved transdermal permeation (Trotta et al., 2004), while others showed little or no effect (El Maghraby et al., 2001; Trotta et al., 2002).

However, for a lipophilic drug, the penetration of the drug into the stratum corneum associated with (solubilized in) vesicular lipid bilayers, bypassing the primary barrier for drug permeation, is expected to have a more important role in improving both skin deposition and transdermal permeation (possibly due to higher solubility in lipids of the stratum corneum and hence relatively

faster release). Penetration enhancing mechanism might also play a role in the enhanced skin delivery of lipophilic drugs; however, it is not the main factor operating (El Maghraby et al., 2000b). Therefore, lipophilic drugs should be entrapped in vesicles for optimum skin delivery. Based on these findings, highly deformable carriers need to be designed and tested on a case by case basis. Further studies, using different hydrophilic and lipophilic agents, are necessary to prove this suggestion.

Ethosome is a novel vesicular carrier, recently developed by Touitou et al., showing enhanced skin delivery. The ethosomal system is composed of phospholipid, ethanol and water (Touitou et al., 2000). Although the exact process of drug delivery by ethosomes remains a matter of speculation (Dayan and Touitou, 2000), most likely, a combination of processes contribute to the enhancing effect (Touitou et al., 2000). Ethanol is a well known permeation enhancer (Williams, 2003). However, previous studies (Dayan and Touitou, 2000; Touitou et al., 2000) that compared permeation enhancement of drugs from ethosomal systems versus hydroethanolic solutions showed that permeation enhancement from ethosomes was much greater than would be expected from ethanol alone. A synergistic mechanism was suggested between ethanol, vesicles and skin lipids (Touitou et al., 2000). Ethanol may provide the vesicles with soft flexible characteristics which allow them to more easily penetrate into deeper layers of the skin. It was also proposed that phospholipid vesicles with ethanol may penetrate into the skin and influence the bilayer structure of the stratum corneum (Kirjavainen et al., 1999b) and this may lead to enhancement of drug penetration.

In the current study, KT solution in 30% ethanol showed a slight, non-significant ($P > 0.05$) reduction in cumulative KT permeated and skin deposited after 24 h relative to aqueous control (Table 1). This could suggest that the penetration enhancing effect of ethanol is not an operating mechanism in the observed enhancement of skin delivery of KT by ethosomes. Based on the current study findings, it is suggested that KT should be incorporated in ethosomal vesicles for optimum skin delivery, under non-occlusive conditions. Ethosomes were not able to improve skin delivery of non-entrapped KT. The proposal that ethanol may increase the flexibility of the vesicles, allowing them to more easily penetrate into deeper layers of the skin, could be supported. This may be followed by structural changes in deep layers of the stratum corneum, with subsequent enhancement of drug penetration. The significant greater skin deposition produced in case of vesicles having KT both inside and outside over vesicles having KT only inside or only outside might be due to that the former are not under osmotic stress and will, therefore, transfer themselves more easily into the stratum corneum (Verma et al., 2003a).

5. Conclusion

Based on the current study and previous studies findings, we suggest that both the penetration enhancing effect and the intact vesicle permeation play a role in the enhanced skin delivery of drugs by deformable liposomes, under non-occlusive conditions. The penetration enhancing effect appeared to be of greater importance in the enhanced skin delivery of KT by deformable liposomes, under non-occlusive conditions. However, the exact mechanism by which deformable liposomes could improve skin delivery of drugs might vary according to the physico-chemical characteristics of the drug. Highly deformable carriers must be designed and tested on a case by case basis.

Regarding ethosomes, results suggested that KT should be incorporated in ethosomal vesicles for optimum skin delivery. Ethosomes were not able to improve skin delivery of non-entrapped KT. Ethosomes, having KT both inside and outside the vesicles, showed superior skin deposition.

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