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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.,