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Review Article

Can drug-bearing liposomes penetrate intact skin?

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Abstract

Using liposomes to deliver drugs to and through human skin is controversial, as their function varies with type and composition. Thus they may act as drug carriers controlling release of the medicinal agent. Alternatively, they may provide a localized depot in the skin so minimizing systemic effects or can be used for targeting delivery to skin appendages (hair follicles and sweat glands). Liposomes may also enhance transdermal drug delivery, increasing systemic drug concentrations. With such a multiplicity of functions, it is not surprising that mechanisms of liposomal delivery of therapeutic agents to and through the skin are unclear. Accordingly, this article provides an overview of the modes and mechanisms of action of different vesicles as drug delivery vectors in human skin. Our conclusion is that vesicles, depending on the composition and method of preparation, can vary with respect to size, lamellarity, charge, membrane fluidity or elasticity and drug entrapment. This variability allows for multiple functions ranging from local to transdermal effects. Application to dissimilar skins (animal or human) via diverse protocols may reveal different mechanisms of action with possible vesicle skin penetration reaching different depths, from surface assimilation to (rarely) the viable tissue and subsequent systemic absorption.

Introduction

Transdermal drug delivery has many potential advantages over other routes of administration including the avoidance of gastrointestinal tract problems and hepatic first pass effects and improvement in patient compliance. Unfortunately the barrier nature of the skin makes it difficult for most drugs to enter by this route (Barry 1983).

Numerous techniques have been employed to improve transdermal delivery (Barry 2001; Williams 2003). These include the use of chemical penetration enhancers (Goodman & Barry 1988; Williams & Barry 2004), adjustment of chemical potential of the drug (Megrab et al 1995), electrically driving molecules into or through the tissue employing iontophoresis (Miller et al 1990) or physically disrupting the skin structure, for example by electroporation or sonophoresis (Prausnitz et al 1993, 1996; Kost et al 1996). Vesicular drug delivery systems such as liposomes, niosomes, ethosomes and transfersomes provide an alternative for improved skin drug delivery (Mezei & Gulasekharam 1980; Cevc & Blume 1992; Schreier & Bouwstra 1994; El Maghraby et al 1999; Touitou et al 2000a, b).

Liposomes are vesicles in which one or more lipid bilayers entrap an aqueous volume. Their major components are usually phospholipids with or without cholesterol. Transfersomes (ultradeformable vesicles) are structurally similar to liposomes but they differ in function (see below); again phospholipids are the major components but an additional surfactant acts as an edge activator to modify elasticity and increase deformability. Ethosomes are phospholipid vesicles, which include ethanol to increase the elasticity, whereas niosomes comprise surfactants together with cholesterol and may include small proportions of phospholipids.

The functions of vesicles as skin drug delivery systems are controversial with variable effects being reported. They can act as drug carriers controlling release of the therapeutic agent. Alternatively they may provide a localized depot in the skin so reducing the amounts of drug permeating through the skin thus minimizing systemic effects. They may also provide targeted delivery to skin appendages and vesicles can enhance transdermal drug delivery, increasing systemic drug concentrations. Indeed, some vesicles may possess several of the above attributes, with the main function differing with application procedure. With such variety of function, it is not surprising

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that the mechanisms of action of these nano-structures as skin drug delivery systems are contentious. Accordingly, the aim of this article is to provide a critical review of vesicles as delivery systems with emphasis on their potential mechanisms of action. For space reasons the text will not provide details on preparation and characterization of vesicles but the effect of composition and elasticity on penetration will be considered. The combination of vesicles with other enhancement techniques, such as electrical methods, has been reviewed elsewhere (e.g. Bonner & Barry 2005, 2006; Essa et al 2005).

Vesicles as skin drug delivery systems

Initially we have reviewed the functions of liposomes irrespective of their mechanisms of action. As they have been used for different purposes, we have first considered their value in localizing drugs within the skin at the site of action, where the goal is to reduce systemic absorption and thus minimize side effects. Secondly, examples where vesicles have been employed for targeted delivery to skin appendages have been considered before examining their potential for improving transdermal delivery (i.e. increased systemic absorption).

Localizing effects

Localization of therapeutic agents within the skin was reported in the first ever publication on liposomes as skin drug delivery systems. In that work, vesicles of dipalmitoylphosphatidylcholine (DPPC) and cholesterol (CH) (1.1:0.5, molar ratio) increased the concentration of triamcinolone acetonide in the epidermis and dermis by 4–5-times and reduced percutaneous absorption compared with a standard ointment (Mezei & Gulasekharam 1980). When the same drug in the identical liposome formulation was prepared in a gel dosage form, and was tested against a gel containing free drug and the nano-aggregate components at the same concentrations, similar findings were observed (Mezei & Gulasekharam 1982). This initial publication emphasized the importance of liposomal encapsulation and hence good entrapment efficiency of the drug in the vesicle for efficient skin drug delivery. Similar findings were reported for progesterone and econazole delivered from liposomes with a lipid composition of DPPC and CH (2:1), applied to guinea-pigs and compared with conventional dosage forms (Mezei 1985). Topical input from similar nano-structures (DPPC, CH; 1.1:0.5) encapsulating 5α-dihydrotestosterone was inferior to that from an acetone solution containing the same drug concentration with respect to increasing the size of the flank organs of the female hamster (Vermorken et al 1984). These results were considered contrary to Mezei & Gulasekharam's early findings and the authors attributed the discrepancy to four possible reasons: firstly, the use of different steroids; secondly, Mezei & Gulasekharam used skin deposition as a measure for activity whereas the Vermorken group depended on the biological effect; thirdly, the use of different animals; and finally, the schemes of application were different. However, it may be noted that both groups used equal drug concentration rather than equal thermodynamic activity for their controls. In addition, the biological effect on the hamster flank organs may have required systemic drug delivery rather than a liposome-mediated localized effect.

The deposition of hydrocortisone into human skin was significantly higher when inserted from phosphatidylcholine (PC)/CH liposomes compared with an emulsion ointment form. The blanching effect of hydrocortisone paralleled the deposition results, with the nano-aggregates producing a greater effect as contrasted with the ointment even when urea was incorporated into this base. Pharmacokinetic studies using guinea-pig confirmed further the localizing effect of liposomes as evidenced by the lower serum level and urinary excretion of the drug obtained after topical application of liposomes, evaluated against ointment (Wohlrab et al 1992).

Using a cutaneous herpes simplex virus guinea-pig model, topical delivery of the peptide drug interferon from liposomes was superior to that from a water-in-oil emulsion or aqueous solution, as revealed by reduced lesion scores after liposomal application. Interferon delivery from liposomes made from the stratum corneum lipids (SCL) produced greater remission of lesions than when input from phospholipid liposomes. With respect to the method of nano-aggregate preparation, those produced by a dehydration rehydration (DRV) method were more effective than when prepared by mechanical shaking or extrusion (Weiner et al 1989). The authors attributed the superiority of DRV liposomes to increased drug partitioning into the vesicle, but the advantage could also have been due to raised entrapment efficiency. In a subsequent study employing guinea-pig skin in-vitro, drug deposition in the tissue was determined using a tape stripping technique and the amount of interferon permeated was also evaluated. Liposomes increased the deposition of interferon into stratum corneum and deeper skin strata with no drug detected in the receptor. Again SCL nano-structures prepared by DRV were considered superior to other formulations (Egbaria et al 1990a). This study indicated that the pharmacological effect obtained by Weiner et al (1989) was due to improved skin accumulation (i.e. a localizing effect). The build up of ciclosporin in the stratum corneum of hairless mice from various liposomal formulations was in the order of SCL liposomes > phospholipid liposomes > o/w emulsion (emulsifier composition similar to lipid content of phospholipid vesicles) > hydroalcoholic drug solution (5 mg mL^{-1} in 40% ethanol). Further, transdermal absorption was reduced from the nano-structure formulations as compared with the solution form (Egbaria et al 1990b). This series of studies reflected the localizing effects of liposomes and highlighted the dependence of deposition 'efficiency' on the lipid composition and method of preparation. SCL and phospholipid liposomes have also been evaluated as skin delivery systems for corticosteroids. Both types of nano-aggregates showed better skin deposition for various drugs when compared with an ointment form, and again SCL vesicles were better than other liposomal formulations. As with the evaluation of Wohlrab et al (1992), the anti-inflammatory effect from nano-structural delivery paralleled the skin accumulation results (Fresta & Puglisi 1997).

The potential of liposome-encapsulated local anaesthetics to provide topical anaesthesia to intact skin was investigated. Using the pin-prick assay, prolonged anaesthesia from a tetracaine nano-formulation was shown, whereas a cream control formulation was ineffective (Gesztes & Mezei 1988). Using the same assay, liposomes prolonged lidocaine anaesthesia compared with a conventional cream. Monitoring drug tissue levels revealed a higher deposition in the epidermis and dermis after application of liposomes than from the cream (Foldvari et al 1990), indicating that the improved anaesthetic effect after vesicle delivery was probably due to improved skin accumulation. Both increased drug permeation through, and deposition into, human skin were recorded in-vitro for liposomal tetracaine relative to an ointment containing the same drug concentration (Foldvari 1994). Vesicles provided even stronger and deeper anaesthesia relative to a commercial eutectic mixture of local anaesthetics (EMLA, 2.5% lidocaine and 2.5% prilocaine) in man (Hung et al 1997; Fisher et al 1998).

In an in-vitro experiment using rat skin, the delivery of triamcinolone acetonide from different liposomes was investigated (Yu & Liao 1996). Nano-encapsulation facilitated the retention and permeation of triamcinolone acetonide compared with the ointment form. This finding was contrary to the early reports of Mezei & Gulasekharam (1980, 1982) who showed improved skin deposition and reduced permeation, and also differed from subsequent studies such as those of Egbaria et al (1990a) and Fresta & Puglisi (1997) who also reported a localizing effect of skin lipid vesicles. This discrepancy could have been due to the use of different membranes; permeability differences between species and especially the relatively poor correlation seen between rat skin as used by Yu & Liao (1996) and human skin can make direct comparisons between laboratory protocols problematic. In addition to steroids, the localizing effects of liposomes have also been sought for treatment of various skin conditions such as psoriasis. Thus, radiotracer studies in mice revealed improved skin build up of tacrolimus from a vesicle lotion compared with intravenous injection of the solution or liposomal formulation (Erdogan et al 2002). In addition to the 9-fold increase in skin levels of the drug following topical nano-aggregate delivery compared with systemic delivery, the vesicular formulation also prevented delayed-type hypersensitivity reactions seen with systemic provision.

The above studies all employed traditional liposomes made of phospholipids or skin lipids. Planas et al (1992) reported an improved anaesthetic effect of lidocaine and tetracaine when inserted from deformable vesicles (transfersomes). In-vivo studies on rats employed the tail-flick assay (response to heat) and the pressure sensitivity of the nociceptors on the tails, to assess anaesthesia after input via transfersomes (PC, sodium cholate), liposomes (PC) and drug solution. For studies in man, the pinprick method was adopted to evaluate the pharmacological effect. It is important to note that the authors applied the tested formulations under occlusion for 25 min, although this application protocol was contrary to the recommended open application for transfersomes. Transfersomes produced enhanced anaesthesia compared with drug solution or traditional liposomes. Surprisingly, topically-applied anaesthetic transfersomes generated an effect equivalent to that created after subcutaneous injection of the same formulation.

Using heat-separated human abdominal epidermal membranes, El Maghraby et al (2001a) investigated the skin delivery of 5-fluorouracil from similar transfersome formulations when applied as a finite dose. The study used saturated aqueous preparations and employed aqueous or water/ethanol (50% v/v) receptor solutions. The results (Figure 1) revealed a marginal increase in transepidermal input with the aqueous receptor. However, the ethanolic receptor significantly increased drug permeation when using the vesicles. These findings indicated improved deposition of the drug in the skin as ethanol was expected to diffuse into the tissue disrupting deposited vesicles and extracting the drug. Additionally, Cevc et al (1997) reported that distribution from transfersomes was dose dependent; the use of finite or infinite doses could vary the relative proportion of localized to transdermal drug amounts and hence optimization of the applied dose was an important facet of transfersomal action.

Targeted delivery to skin appendages

Although most reports into the localizing effects of liposomes have concentrated on drug deposition into the stratum corneum and viable epidermis, several workers have studied the potential of such nano-structures for targeting the appendages, especially to the pilocebaceous units (hair follicles with their associated sebaceous glands). This area was extensively reviewed by Lauer et al (1996) and Lauer (1999).

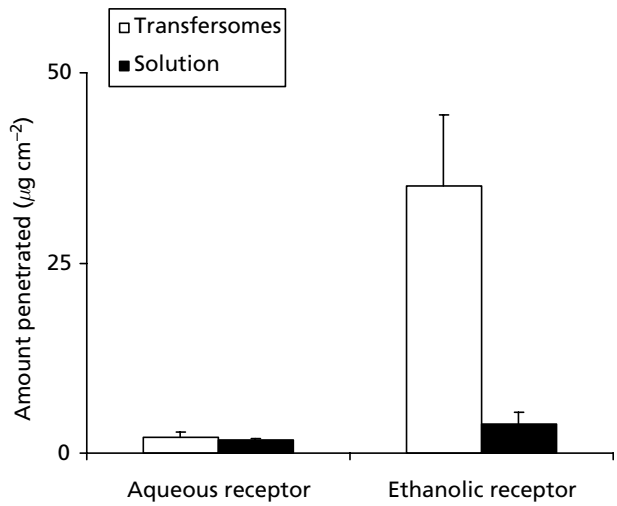

Figure 1 Amount of 5-fluorouracil penetrated through human epidermis 36 h after application of transfersomes or a saturated aqueous solution.

Employing the hamster ear model, liposomes of PC, CH and phosphatidylserine (PS) selectively targeted the fluorescent hydrophilic dye, carboxyfluorescein, into the pilosebaceous units. Liposomes showed better targeting than aqueous solutions even when these solutions contained 10% ethanol or 0.05% sodium lauryl sulfate or when propylene glycol was the donor vehicle (Lieb et al 1992a, b).

The deposition of γ-interferon from aqueous solution and from PC, CH and PS vesicles containing the same amount of drug into human skin and the skin of hairless mice and hamster was compared in-vitro. Within the three species, vesicles improved input compared with aqueous solutions. The greatest accumulation was seen in hamster skin, which also possessed the highest follicular density. It was thus concluded that the follicular pathway could be a route for drug deposition from liposomes (Du Plessis et al 1992).

Significant amounts of cimetidine were placed into the pilosebaceous glands and other skin strata of the Syrian male hamster ear, after topical application of the drug in 50% aqueous ethanol, nonionic liposomes (niosomes), or in phospholipid liposomes. Surprisingly, both an alcoholic solution and a nonionic vesicular formulation were pharmacologically active, producing an equal effect, but the phospholipid nano-arrangements were ineffective, when probed by monitoring the reduction in the size of sebaceous glands (anti-androgenic effect). The authors explained the lack of a biological effect from the phospholipid vesicles, despite increased drug provision into the tissue, on the basis that the negative charge of lipids could form an ion pair with cimetidine at pH 5.5 (the pH of the formulation). Cimetidine thus accumulated as an inactive ion pair (Lieb et al 1994). Autoradiography revealed the presence of considerable amounts of caffeine in the appendages after topical application of liposomes to rat skin but most of the drug was localized in the epidermis (Touitou et al 1994).

The sebaceous-gland deposition of isotretinoin from a liposomal gel formulation of soya-PC (SPC), mixed micelles in gel (SPC, sodium glycocholate) and an ethanolic gel was monitored using human facial skin. Contrary to the above reports, neither liposomes nor mixed micelles provided any advantage over the ethanolic gel with regard to follicular delivery. This finding was attributed to the highly lipophilic nature of the drug which would intrinsically target the sebaceous gland (Tschan et al 1997). However, considering that ethanol is a solvent used to enhance follicular delivery through partial solubilization of the sebum or softening of the material in the duct, the selection of the solvent could account for enhanced input of the drug from the ethanolic control with the other formulations then shown to be equivalent to the control. Whilst these findings could suggest a positive effect of liposomes and mixed micelles, we can only conclude that they were as effective as the ethanolic gel.

Vesicular preparations were found superior in the treatment of acne vulgaris compared with conventional preparations including alcoholic lotions (Meybeck 1992; Skalko et al 1992). Again, and in accord with the majority of literature in the field, this is strong evidence that vesicles can effectively target drug delivery to skin appendages.

Improved transdermal delivery

Although the majority of reports dealing with standard liposomes concentrated on improved drug deposition into skin and its appendages, some early sources cited improved transdermal delivery from these nano-aggregates. According to the results of permeation experiments involving finite dose applications to hairless mouse skin, Ganesan et al (1984) reported that, for lipophilic drugs, greater amounts may be input from vesicles compared with aqueous solution. Qualitative immunohistochemical examination, which involved staining of vertical sections of the skin (stratum corneum, epidermis, dermis and subcutis), revealed that liposome-encapsulated antibodies (with molecular weight 20 000–50 000 Da) distributed rapidly into the deep cutaneous regions of piglet skin in-vivo, compared with no penetration after application of aqueous solution or empty vesicles. Quantitative studies using radiolabelled markers revealed not only improved deposition into the deep strata but also a clearly raised percutaneous absorption from nano-aggregates compared with aqueous solution (Artman et al 1990a, b). Formulations containing unilamellar soya-lecithin/CH liposomes advanced the percutaneous absorption of methyl nicotinate compared with aqueous solution or gel formulations (Bonina et al 1995). Fresta & Puglisi (1996) found that vesicles containing Epikuron 200, a phospholipid with unsaturated alkyl side chains (fluid liposomes), produced high percutaneous absorption and tissue distribution rather than skin accumulation. Renal elimination of inulin was 20-fold higher after usage of such liposomes compared with aqueous solution. A liposomal formulation of PC and CH augmented the uptake of biologically-active recombinant human γ-interferon into the epidermis of viable human skin compared with aqueous solution (Short et al 1996). This study employed a human skin graft model and monitored the expression of intercellular adhesion molecule-1 by light level immunomicroscopy, assessing the biological effect.

While researchers were reporting mainly localized or rarely transdermal effects of liposomes, Cevc & Blume (1992) claimed that certain types of lipid vesicles (transfersomes) could penetrate intact to the deep layers of the skin and might progress far enough to reach the systemic circulation. Importantly, they stated that, for successful delivery, transfersomes must be applied under non-occlusive conditions. Using radiolabelled lipid components, the fate of the transfersomes was followed after occlusive and non-occlusive application, and was compared with that of traditional liposomes (Cevc & Blume 1992). The use of liposomes or occluded transfersomes resulted in approximately 25% of the dose being in the stratum corneum, a very few percent in the deeper layers of the epidermis, but most of the dose was recovered from the skin surface. However, with non-occluded transfersomes treatment, 30% of the dose was found in the subdermis and up to 6–8% was detected in the blood. This indicated the superiority of deformable vesicles over the standard liposomes

for transdermal drug delivery. It also demonstrated the importance of open application of these deformable nano-aggregates, although a deviation from this protocol can be found (Planas et al 1992), where an improved anaesthetic effect was reported after occluded treatment with anaesthetic transfersomes. However, it may be noted that even for standard liposomes, occlusive dosing was used in the above studies assessing local anaesthesia. Transdermal immunization with large proteins by means of transfersomes has also been achieved (Paul et al 1995). Further, transfersomes were reported to improve the regio-specificity and the biological activity of the corticosteroids hydrocortisone, dexamethasone and triamcinolone acetonide, in-vivo. The effect was dose-dependent and it was concluded that this carrier can target the drug into the viable skin and when used in a higher dose, can distribute the medicament throughout the body (Cevc et al 1997).

As a test of biological action, the suppression of arachidonic acid-induced oedema on the inner side of mouse ear was adopted. Transfersomes provided oedema suppression equivalent to a lotion containing 5-times the drug concentration of that in deformable vesicles, after 0.5 h. Subsequently, after 2 h, the transfersome formulation was more efficacious than the lotion. When standard nano-carriers (PC, cholesterol; 1:1, molar ratio) were evaluated, no oedema suppression was found after 0.5 h. After 2 h, however, liposomes produced a measurable suppression. The data showed that the effect of liposomes (after 2 h) was approximately one third that of deformable vesicles and approximately half that of the lotion (with 5-times more drug). The authors stated that the late effect of the vesicle formulation arose from free drug permeation following its release from liposomes (Cevc et al 1997). However, if this explanation is valid, vesicles would be expected to provide one fifth of the efficacy of the lotion (containing free drug), unless there is some penetration enhancing effect for such liposomes.

Successful systemic delivery of insulin has been achieved by transfersomes as reported from in-vivo mice and human studies. Animal investigations revealed the transfer of [^{125}I]insulin across intact skin with subsequent distribution throughout the body after external application of transfersomes. The biological studies (mice and man) indicated that topically applied insulin transfersomes reduced blood glucose levels. The efficiency of the formulation was comparable with that obtained after subcutaneous injection of the same preparation but with a longer lag time. This lag time may be required for vesicle skin penetration. Both mixed micelles and liposome formulations of insulin failed to reduce blood glucose. It was thus concluded that transfersomes provide non-invasive transdermal delivery of therapeutic agents, including insulin (Cevc et al 1993, 1995, 1998). In addition, the arachidonic acid-induced oedema suppression test (acute murine ear oedema model) was used to evaluate the anti-inflammatory effect of Cu, Zn-superoxide dismutase after topical application in transfersomes, mixed micelles or liposomes. Of all the tested carriers only transfersomes significantly reduced the oedema (Simoes et al 1998).

In a series of studies involving an optimized experimental design, El Maghraby et al (1999) investigated estradiol skin delivery from a variety of liposomes. The experiments probed optimized ultradeformable vesicles (El Maghraby et al 2000a) relative to standard liposomes (formulation details in Table 1). The ultradeformable formulations included PC with sodium cholate (UD1), PC with Span 80 (UD2) and PC with Tween 80 (UD3). The standard nano-carriers encompassed pure PC vesicles (non rigid, SL1), PC with CH (membrane stabilized, SL2), and two rigid vesicles of DPPC (SL3) and DPPC/CH (SL4). The studies involved low dose open application of the formulations to human epidermal membrane hydrated by an 'open hydration' protocol that maintained the transepidermal water gradient. Permeation experiments were performed in two stages; the first employed an aqueous receptor solution for 12 h, at the end of which the preparations were removed from the donor and the receptor was then changed to 50% aqueous ethanol for a further 12 h (second stage). This design provided the permeation parameters (maximum flux and its time) from the first section and measured the skin deposition in the second stage. The results (Figure 2) indicated that all types of

Table 1 Codes, composition and types of the liposome formulations

Code	Composition	Type
UD1	PC, sodium cholate; 86:14 (w/w)	Ultradeformable
UD2	PC, Span; 86.7:13.3 (w/w)	Ultradeformable
UD3	PC, Tween; 84.5:15.5 (w/w)	Ultradeformable
SL1	Pure PC	Non rigid
SL2	PC, cholesterol; 1:1 (molar ratio)	Membrane stabilized
SL3	Pure DPPC	Rigid
SL4	DPPC, cholesterol; 2:1 (molar ratio)	Rigid

UD, ultradeformable; SL, standard liposomes; PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine. UD vesicles contained 7% v/v ethanol in the final formulation.

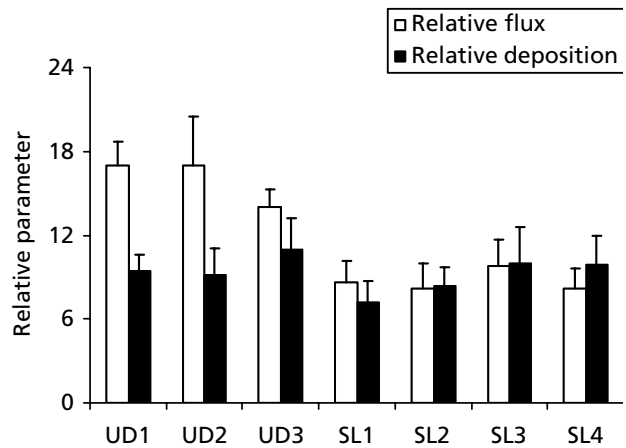

Figure 2 Estradiol flux through, and deposition into, human epidermis after application of ultradeformable (UD) or standard (SL) liposomes in-vitro. Data are presented relative to saturated aqueous control.

liposomes improved both estradiol deposition into and permeation through the epidermis compared with the saturated aqueous control. The ultradeformable vesicles were better than the standard liposomes with respect to transepidermal drug flux but there were no significant differences between different types of nano-carriers with regard to estradiol accumulation in the skin. The ultradeformable liposomes reduced the time of maximum flux (T_{max}) by 11–16%. For the standard liposomes, T_{max} was either constant (SL1) or increased by 10–20% (SL2–4).

The presence of a surfactant in liposomes increases the fluidity (flexibility) or elasticity of the lipid bilayers (El Maghraby et al 2004). Consequently, it can be concluded that flexible liposomes are more efficient in delivering drugs across the epidermis. It was suggested that such surfactants (edge activators) can impart deformability to the nano-carriers, which allows for improved transdermal drug delivery (Cevc 1992, 1995, 1996). The incorporation of ethanol in lipid vesicles (ethosomes) is an alternative approach to fluidize the lipid membrane and thus enhance drug provision (Touitou et al 2000a, b). Also flexible vesicles with other surfactant components showed higher efficiency compared with rigid vesicles (Honeywell-Nguyen et al 2003a, 2004).

Mechanisms of action of liposomes as skin drug delivery systems

Alternative mechanisms have been suggested for liposomes acting as delivery systems (Figure 3). Scheme A represents a free drug operation whereby molecules initially release from liposomes followed by independent permeation through skin, or via direct carriers/skin drug exchange by 'collision complex transfer' between drug intercalated in the liposomal bilayer and the surface phase of the stratum corneum through a thin hydrophilic film at the interface. Both mechanisms may combine (Ganesan et al 1984). This report excluded intact liposome skin penetration or vesicle fusion to the skin surface. Ultrastructural changes in the stratum corneum have been reported after skin was treated with nano-carriers containing high PC concentrations, suggesting a possible penetration enhancement effect—mechanism B (Hofland et al 1995). The processes of adhesion onto the skin surface (C), with possible fusion or mixing with the lipid matrix of stratum corneum have been suggested for liposome lipids (Kirjavainen et al 1996). On the basis of electron microscopic observations, using colloidal iron as a marker, it was proposed that traditional vesicles of DPPC, cholesterol (2:1) can penetrate into skin to act as depot (Foldvari et al 1990). Shortly after this suggestion, transfersomes were reported to penetrate intact into and through skin—process D (Cevc & Blume 1992). In the subsequent sections we will consider the proposed different mechanisms, illustrating both positive and negative findings.

Free drug mechanism (see Figure 3 at A)

According to this process, the drug permeates the skin independently after exiting from the vesicles (Ganesan

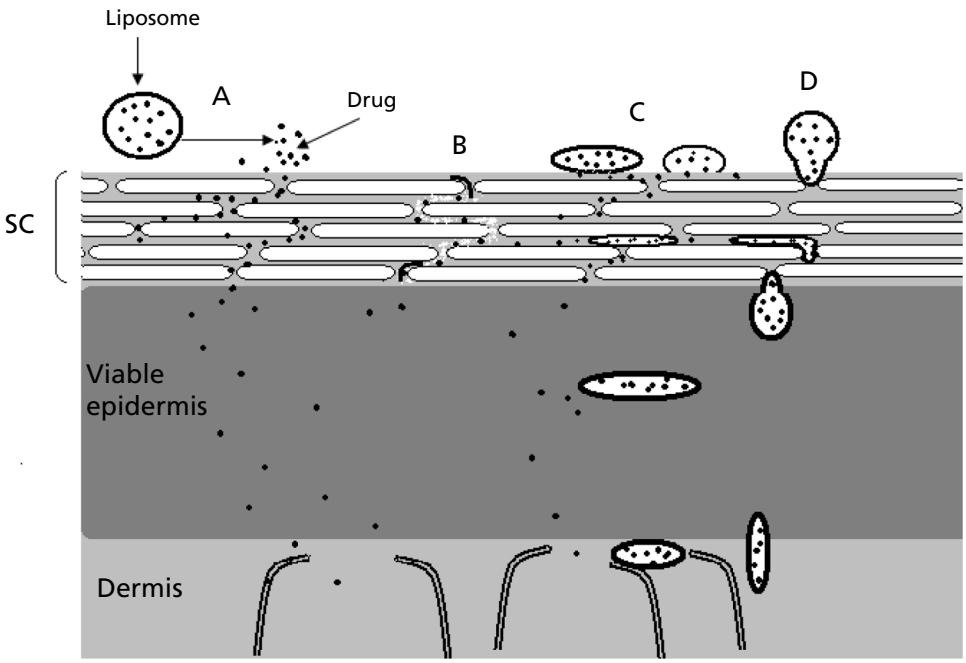

Figure 3 Possible mechanisms of action of liposomes as skin drug delivery systems. A. Drug free mechanism. B. Penetration enhancing process of liposome components. C. Vesicle adsorption to and/or fusion with the stratum corneum (SC). D. Intact vesicle penetration into or into and through the intact skin (not to scale).

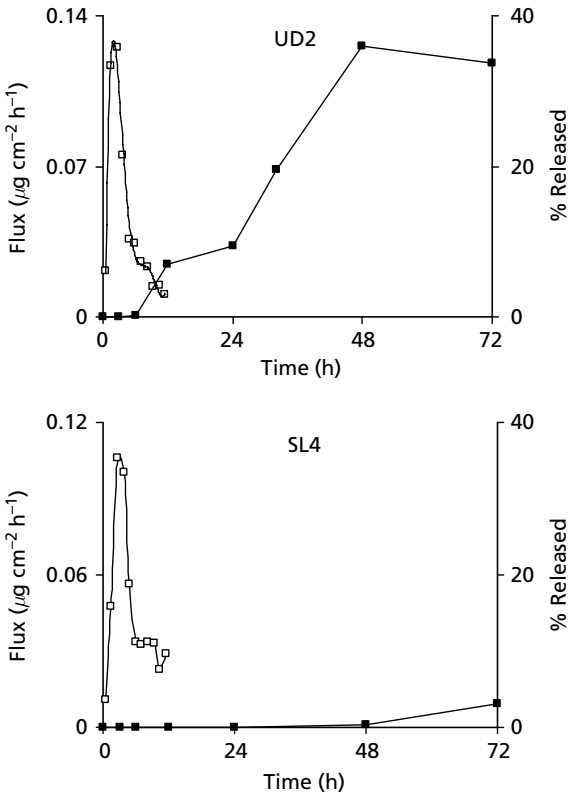

Figure 4 Example plots of correlation between the estradiol transepidermal flux (open symbols) and its in-vitro release (closed symbols) from liposomes (after El Maghraby et al 1999).

et al 1984). Such vesicles can be considered only as carriers that can control drug release. To investigate this possibility, we compared the transepidermal flux plot of each individual formulation with its in-vitro drug release profile (see e.g. Figure 4). For all preparations, the peak flux of estradiol through skin appeared at a time during which drug release was negligible. This suggested that a free drug mechanism did not operate for any of the formulations (El Maghraby et al 1999).

Penetration enhancing mechanism (see Figure 3 at B)
Kato et al (1987) first recorded a penetration enhancing effect for egg lecithin (included in a drug solution in propylene glycol) after in-vitro and in-vivo animal studies. They concluded that lecithin enhanced the transdermal delivery of bunazosin hydrochloride by lowering the permeability barrier of the skin. This early finding suggested the possible accelerant effect of a liposome component.

After an in-vivo study predicting the steady-state flux of methyl nicotinate from the rate of drug disappearance from a donor compartment applied to human arm (difference method), it was found that incorporation of phospholipone 80 in the vehicle enhanced the skin penetration of the drug. This acceleration was explained on the basis that the stratum corneum lipids can be solubilized by the inverse micelles formed from the phospholipids (Leopold & Lippold 1995).

Freeze-fracture electron microscopy and small angle X-ray scattering studies, performed after dipping human stratum corneum in a liposome suspension for 48 h, revealed that vesicle components can change the ultrastructure of the intercellular lipid regions. This suggested a penetration enhancing effect. It was concluded that nano-aggregates containing relatively small hydrophilic head groups showed marked interaction with human stratum corneum in-vitro (Hofland et al 1995).

Another finding that supported a sorption enhancing mechanism of liposome components was that of Korting et al (1995). After application of large unilamellar liposomes (mainly made from soybean PC) to human epidermis reconstituted in-vitro, electron microscopy revealed the presence of dose-dependent alterations in the morphology of both the stratum corneum and the previously viable epidermis. Shrunken lipid droplets formed between the corneocytes. In addition, both the corneocytes of various layers of the stratum corneum and the keratinocytes of the upper layer of the living epidermis showed lipid deposition.

In another study, Zellmer et al (1995) treated human stratum corneum (non-occlusively) with dimyristoylphosphatidylcholine (DMPC) liposomes, followed by differential scanning calorimetric investigations. DMPC vesicles did not penetrate into stratum corneum but the lipid could penetrate and change the enthalpy of the lipid-related transitions of the stratum corneum. In addition, Kirjavainen et al (1996) revealed that, depending on composition, vesicles may produce an enhancing effect (shown by skin pretreatment), may penetrate deep into the stratum corneum or may fuse and mix with skin lipid. Liposomes containing dioleylphosphatidylethanolamine (DOPE) or lyso-PC produced the greatest effect. They also reported that nano-aggregates incorporating DOPE could fuse and mix with skin lipids and loosen their structure. This was evidenced by the interactions of these vesicles with stratum corneum lipid liposomes. It was thought that the conical shape of DOPE was essential for this effect. Both the PE and dioleyl moieties were essential, as nano-structures containing PE (with other fatty acid chains) provided lower enhancing effects compared with the DOPE liposomes.

In more recent studies, liposome–skin interactions and their effects on skin permeation of drugs were probed in-vitro (Kirjavainen et al 1999a). The stratum corneum penetration of a lipophilic fluorescent probe was deeper for PC liposomes containing 32% ethanol compared with ethanol-free vesicles. Ethanol did not affect the penetration pattern from DOPE-containing nano-aggregates. However, addition of ethanol increased the mixing of both vesicles with stratum corneum lipid liposomes. In addition, ethanol-containing nano-structures (both types) destabilized skin lipid ones as evidenced by increased calcein release from SCL preparations compared with control (containing the same concentration of ethanol). This indicated that vesicles could have a penetration enhancing effect.

Phospholipid solutions in propylene glycol increased the percutaneous absorption of indomethacin and the order of efficiency was; phosphatidylglycerol (PG) > phosphatidylethanolamine (PE) > PC >

phosphatidylserine (PS) > phosphatidic acid (PA) > phosphatidylinositol (PI) > control > sphingomyelin (Yokomizo & Sagitani 1996a). Also skin pretreatment with PG, PC or PE promoted drug permeation and shortened the lag time. The authors classified the lipids into three groups: PG, PE and PC with head groups having a strong enhancing effect; PS, PA and PI with such groups providing a weak promoting effect; sphingomyelin is not an enhancer. When repeating the same study on silastic membrane instead of skin, no phospholipid accelerated the permeation of the drug. Furthermore, PG, PE, PC or sphingomyelin did not significantly affect the percutaneous absorption of indometacin through skin lacking stratum corneum. This clearly indicated that phospholipids act directly on the permeability barrier of stratum corneum. In a similar study, the effects of the hydrophobic entity in the phospholipids were probed (Yokomizo & Sagitani 1996b). PG, PC and PE derivatives were evaluated. The enhancing consequence of PG derivatives was in the order of DOPG (dioleoyl) > PGE (from egg yolk) > PGS (from soybean) > DMPG (dimyristoyl) > control > DPPG (dipalmitoyl) > DSPG (distearyl). For the PC derivatives it was DOPC > DLPC (dilauryl) > PCS > PCE > HPC (hydrogenated) = control. For the PE derivatives, the ranking was DOPE > PE > control. The enhancing outcome of most phospholipids was even better than for traditional accelerants (fatty acids and Azone). It was suggested that phospholipids containing unsaturated fatty acid chains in the hydrophobic group were strong promoters. As in the previous studies, the authors showed that the phospholipids worked by directly affecting the stratum corneum.

The human skin blanching assay assessed the effect of phosphatidylcholine (PC) on the topical bioavailability of corticosteroid creams (hydrocortisone, clobetasone butyrate, betamethasone and clobetasol propionate). Arms were pretreated twice daily (by open application of liposomes) for seven days before applying corticosteroid. This pretreatment was also performed during corticosteroid application with liposomes applied at least one hour before application. The pretreatment increased the blanching response and reduced the tachyphylaxis for all preparations except clobetasone butyrate. The authors explained this effect on the basis that PC may form a thin film on the skin surface into which corticosteroids can preferentially partition, or PC can partition into stratum corneum and thus enhance by influencing the partitioning of corticosteroid into skin (Jacobs et al 1988).

Foldvari (1994) reported that intact vesicular penetration into skin cannot be the only mechanism responsible for the high rate of tetracaine penetration from liposomes and that an enhancing mechanism was possible. This conclusion followed detection of the radiolabelled phospholipid in skin after application of tetracaine liposomes, but the ratio of lipid to tetracaine was much less than originally in the preparation.

Negative findings concerning an enhancing process also appear in literature reports. Some examples will be summarized here. No accelerant effect was found for SCL liposomes when the empty vesicles were applied in combination with free interferon (Weiner et al 1989). Du Plessis et al (1994a) studied the influence of in-vivo skin pretreatment with liposomes on the topical absorption of hydrophilic (inulin) and hydrophobic (hydrocortisone) drugs. The vesicles included both phospholipid (PC, CH, CS) and skin lipid formulations. They found that pretreatment did not give the advantages of encapsulated drug, showing no enhancement for hydrophilic and lipophilic drugs. They concluded that the hypothesis that liposomes interact with stratum corneum was invalid. It was further suggested that they must at least be applied concomitantly with the drug or the drug must be encapsulated within them.

Employing a pretreatment protocol with empty liposomes, El Maghraby et al (1999) investigated the penetration enhancing effect of different formulations on transepidermal delivery of estradiol. Standard liposomes (SL1–4) and ultradeformable vesicles (UD1–3) were tested. The results indicated a possible accelerant effect only for the non-rigid PC vesicles (SL1) for which drug flux improved 4-fold. However, comparing this promotion with the relative flux obtained after application of estradiol encapsulated in the same formulation (8-fold) it was concluded that enhancement was not the main mechanism operating. For other traditional and ultradeformable systems, skin pretreatment was not effective, thus excluding an accelerant effect and suggesting other mechanisms for the improved skin delivery from such formulations.

Investigating the importance of liposome structure, El Maghraby et al (2000b) compared estradiol skin delivery from standard and ultradeformable nano-aggregates with that obtained from propylene glycol solution containing the same components. All systems were at the same thermodynamic activity. The results indicated the importance of the colloidal structure. These findings again excluded a major role for a sorption promoting mechanism in improved skin delivery from such liposomes.

In a more recent study investigating the efficiency of elastic and rigid vesicles, it was reported that drug molecules must be applied together with and entrapped within the nano-aggregates themselves, suggesting that elastic vesicles act as drug carrier systems and not as penetration enhancers (Honeywell-Nguyen et al 2003a).

Vesicle adsorption to and/or fusion with the stratum corneum (see Figure 3 at C)

The interaction of liposomes with human skin has been reviewed, highlighting the role of the stratum corneum (Schaller & Korting 1996). It was concluded that they can be taken into the skin but cannot penetrate through intact healthy stratum corneum; instead, they dissolve and form a unit membrane structure. Fast penetration of vesicular lecithin into human skin has also been reported (Wohlrab et al 1989). The processes of adhesion onto the skin surface and fusion or mixing with the lipid matrix of stratum corneum have been suggested for liposome lipids (Kirjavainen et al 1996). Investigating vesicular delivery of hydrocortisone, Kim et al (1997) suggested that the phospholipid component of the nano-aggregates could enter the skin rapidly with the steroid following the fate of phospholipid.

Phospholipids increased the partitioning of estradiol, progesterone and propranolol into the stratum corneum lipid bilayers (Kirjavainen et al 1999b). All these findings suggested the possibility that liposomes could improve skin partitioning and thus the uptake of drugs. Accordingly, an uptake study was designed (El Maghraby et al 1999) in which stratum corneum membranes were dipped into the test formulation or aqueous solution for a short time (10 min). The effect of pretreatment with empty vesicles and the deliverance from medicated carriers were studied. Determining the amount of drug in the membrane, the uptake ratio was calculated relative to the corresponding control (Figure 5). It was suggested that the major component of liposomes, phospholipids, increased the continuity of the lipid matrix of the skin and thus facilitated the movement of lipophilic molecules (Keith & Snipes 1982). Based on this suggestion we should expect improved drug uptake from saturated aqueous solution after skin pretreatment with empty vesicles. But results (Figure 5) showed that such a process produced no significant effect on drug uptake from solution, which indicated the necessity of co-application of drug with the nano-structures.

The uptake from medicated vesicles was significantly higher compared with the control. The uptake ratios between the vesicles and solution ranged from 23 to 29 with no significant differences between individual formulations. Comparing uptake ratios for each formulation with the corresponding relative maximum flux values (in the range of 8.2 to 17, Figure 2), the uptake ratio was higher, which could indicate that the uptake from vesicles was greater than that from a saturated solution. Correlating the superiority of deformable nano-aggregates over traditional liposomes in increasing transepidermal flux, with no significant difference found in the uptake ratios at short contact time, suggested that deformable vesicles either improved the diffusion or penetrated deeper in the epidermis, thus allowing more efficient drug clearance.

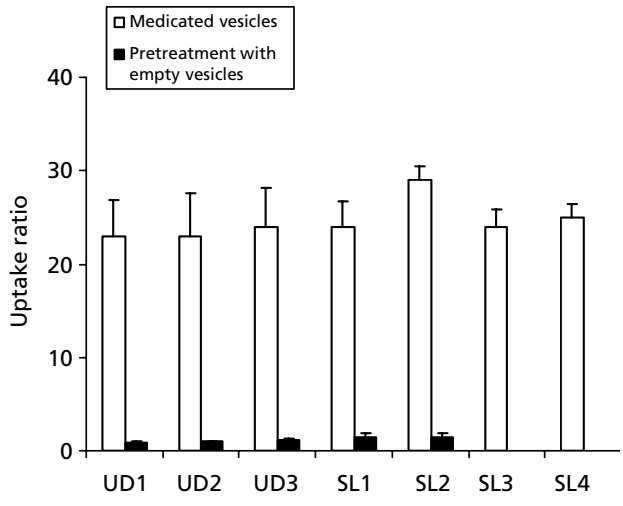

Figure 5 Uptake of estradiol into stratum corneum from aqueous solution (10-min dipping) after pretreatment with different empty lipid vesicles and uptake from the medicated vesicles without pretreatment.

Intact vesicular skin penetration mechanism (see Figure 3 at D)

Traditional liposomes. The concept of intact vesicular skin penetration is not recent and was suggested in the first report on liposomes as skin drug delivery systems. Therefore, we start our consideration from 1980 and 1982 when Mezei & Gulasekharam explained their findings (improved drug deposition in the skin after application in the form of liposomes) on the basis that vesicles penetrated the intact skin carrying the drug. Penetration was reported to reach the vascular dermis. It was suggested that because the liposomes were large they were not able to enter the capillary circulation and thus acted as reservoirs for the drug at the site of action.

Conceptually it was difficult to conceive that large lipid vesicles could penetrate the densely packed stratum corneum in great numbers. Consequently many workers have tested this hypothesis. Foldvari et al (1990) applied DPPC, CH (2:1) liposomes loaded with colloidal iron (an electron dense marker) to guinea-pigs, three times (once every 12 h). Electron micrography showed the presence of intact liposomes in the dermis. Although the applied formulation included large multilamellar vesicles (LMLVs), most of the liposomes detected in the dermis were unilamellar (300–500 nm) with some LMLVs. The authors proposed that liposomes could penetrate the epidermis carrying the drug into skin and that the smaller nano-aggregates could have come from LMLVs that lost their external bilayers during penetration. They added that liposomes could be adsorbed intact on the skin surface before penetration, with a possibility that some vesicles might rupture.

In a clinical investigation, the therapeutic efficiency of betamethasone dipropionate in atopic eczema and psoriasis vulgaris was assessed, comparing liposome gel (egg lecithin, small unilamellar vesicles) with a commercial gel containing propylene glycol (Korting et al 1990). The nano-aggregate formulation was superior in eczema but not for psoriasis. It was thus concluded that vesicles could penetrate diseased skin with its ruptured stratum corneum (as in eczema) but could not invade diseased skin with hyperkeratosis, as in psoriasis. Subsequently it was shown that intact SUVs (PC, CH) penetrated no deeper than the stratum corneum. This was indicated by fluoromicrography of excised human skin after application of liposomes labelled by either lipophilic or hydrophilic fluorophores (Lasch et al 1991).

Using dual-labelled liposome components, the skin deposition of those derived from phospholipid and skin lipid carriers was studied. The ratio of radiolabelled components of liposomal preparations was maintained constant throughout the skin strata. The authors explained this as possible molecular mixing of liposomal bilayers with the stratum corneum bilayers (Egbaria et al 1990a). When [^{14}C]inulin (hydrophilic marker) in liposomes whose lipid bilayer was radiolabelled with [^{3}H]cholesterol was applied, the ratio of inulin to cholesterol was also constant throughout skin strata, with improved inulin deposition. The explanation given by the authors (molecular mixing) would not justify equal ratios of the dual label in the deeper skin strata (below the horny layer). These findings may

suggest possible carrier skin penetration carrying both polar and non-polar drugs.

Similar findings were reported again for both phospholipid and skin lipid liposomes (Fresta & Puglisi 1996). In addition, they monitored the effect of colloidal size on the deposition of components into skin. Unilamellar skin lipid vesicles of approximately 100-nm diameter produced better input into deeper skin strata compared with large MLVs (2.3 μm). This suggested the dependence of skin deposition on vesicle size, which supported the concept of intact vesicular penetration as a possible mechanism for improved skin accumulation via nano-aggregates. Similarly, hydrogenated PC liposomes increased the penetration of tocopherol acetate into skin, with smaller vesicles being superior (Natsuki et al 1996).

Contrary to the previous findings, Du Plessis et al (1994b), who studied the effect of particle size of liposomes on the skin deposition of ciclosporin, found that the intermediate size and not the small size entities resulted in higher amounts. They considered this as an indication that intact liposomes did not penetrate the skin; if such input operated, better results should be obtained as the particle size decreased. Furthermore, no evidence of intact carrier penetration could be found after confocal laser scanning microscopy or electron microscopy performed after application of DMPC or soy-lecithin liposomes (Korting et al 1995; Zellmer et al 1995).

Kirjavainen et al (1996) recorded that improved delivery of a fluorescent marker into deep stratum corneum after vesicular encapsulation was not due to intact penetration. This conclusion was reached after obtaining a delivery of the same marker after skin pretreatment with empty vesicles similar to that obtained from carriers encapsulating the marker. A penetration enhancing effect was the most likely explanation for these results.

The ratio of [^{3}H] DPPC to [^{14}C]tretinoin deposited into various skin strata of the hairless rat after application of PC/CH liposomes was monitored. This ratio was constant throughout the stratum corneum but it was lower than that in the original preparation. In the nucleated epidermis and dermis, the quotient reduced further and decreased gradually with depth in the skin. It was concluded that liposomes and tretinoin co-transport into stratum corneum, accompanied by independent penetration of free drug, which could have escaped from liposomes on the skin surface. Transfer through the epidermis and dermis applied only for the free drug after being released from carriers (Masini et al 1993). This report suggested that vesicles could penetrate only into the stratum corneum.

Monitoring the fate of topically applied liposomes, Short et al (1996) used Nile red dye (uncharged phenoxazine dye) to stain skin strata. The study suggested that at least some of the lipids from PC, CH vesicles entered into and/or transported across the epidermis. However, it is difficult to conclude if any intact entities penetrated as the authors did not address the physical stability of liposomes after topical application.

Ultradeformable vesicles. Transfersomes have been reported to invade the skin intact and to go deep enough to be absorbed by the systemic circulation. This argument was supported by three findings. Firstly, the life time of lipid turnover in phagocytes is approximately 6 h, so in an 8 h experiment there would be insufficient time for degradation of most of the lipid vesicles. This implied that there was little or no monomeric permeation of the lipid components. Secondly, the permeation of lipid-detergent mixed micelles was lower than that of transfersomes. Finally, after skin application, some of the lipid-associated radioactivity was recovered from the liver, which accumulated particles and vesicles but not lipid molecules (Cevc & Blume 1992). The mechanism of transfersomal action was not due to the penetration enhancing effect of the surfactant. This conclusion was supported by a report that increasing cholate concentration above the optimum level reduced the efficiency of these vesicles (Planas et al 1992). In addition, mixed micelles were not as effective as deformable vesicles and it was reported that an optimum surfactant concentration in liposomes was required. In optimized formulations, the additive (e.g. surfactant) concentration was typically 10–60% of that required to dissolve the bilayers (Cevc 1995). The studies of El Maghraby et al (2000a) supported the same argument, with too low or too high surfactant concentration being less effective.

A water concentration gradient develops across the skin. At the epidermal surface the concentration is often less than 15% but increases deeper into the skin to reach five-times the surface value at the basal skin layers (Warner et al 1988). This transdermal hydration gradient is said to produce a force sufficient to drive the ultradeformable lipid vesicle (transfersome) through the intact stratum corneum and into the epidermis. Phospholipid hydrophilicity leads to xerophobia (tendency to avoid dry surroundings) which causes the vesicles to resist dehydration at the skin surface. Accordingly, for the vesicles to remain maximally swollen, those on the skin surface try to follow the local hydration gradient, moving into deeper skin strata (Cevc 1992; Cevc & Blume 1992; Cevc et al 1995). For transfersomes, the water gradient was sufficient to drive them through the skin but for standard liposomes this gradient was insufficient. This was because normal liposomes required a high energy cost to deform (Cevc et al 1993). In support of their hypothesis, they reported that elimination of the water concentration gradient by occlusive application made transfersomal delivery similar to that of traditional liposomes. In addition, the chemical gradient of lipid was not effective, as evidenced by the lack of a proportional relation between the applied mass and permeation (Cevc & Blume 1992). Surprisingly, the same group applied local anaesthetic transfersomes by occlusion under watertight wrapping for 25 min (Planas et al 1992).

The process of transfersome skin penetration was attributed to the high deformability of the vesicle (Figure 6). The reason for the flexibility of this type of carrier is that the edge activator molecules (e.g. surfactants) tend to accumulate at the site of high stress due to their raised propensity for greatly curved structures. This rearrangement reduces the energy required for deformation. The stress is reportedly produced upon drying of the vesicles

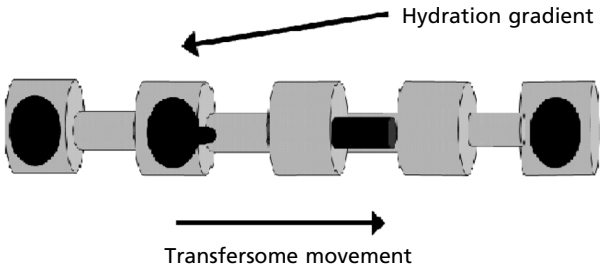

Figure 6 Hydration gradient and deformability-driven movement of transfersomes through small pores (after Cevc et al 1996).

which, being flexible, can follow the transdermal hydration gradient (Cevc et al 1995).

Those authors claimed that an intact vesicular penetration process was the primary mechanism operating. For example, they reported that successful topical delivery of insulin from transfersomes could not be through shunts as they found no significant differences between animals or man with different hair follicle densities. They added that this delivery could not be through micro-lesions in the skin, as they would then expect increased delivery from other carriers (mixed micelles and liposomes), but this was not the case (Cevc et al 1998).

To test this supposition, the epidermal permeation of estradiol from large multilamellar vesicles (LMLVs, at least 557 nm in diameter) was compared with that obtained from smaller entities of a mean size of 124–138 nm (SUVs). The concept was to investigate the possibility that intact nano-aggregates penetrated through skin, assuming that this infiltration was a function of the vesicle size (El Maghraby et al 1999). The SUVs were less than the maximum dimension reported to enter skin and the minimum size of LMLVs was above the maximum volume which can invade skin (Cevc et al 1995). Standard and ultradeformable formulations were evaluated. The relative fluxes of estradiol delivered from SUVs and LMLVs are shown in Figure 7. Comparing the relative J_{max} values

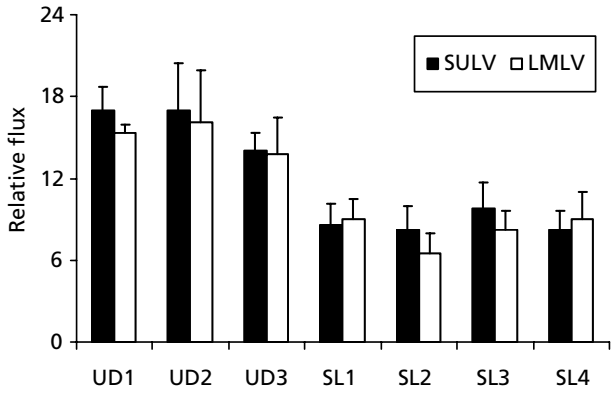

Figure 7 Effect of vesicle size and type on the transepidermal delivery of estradiol from liposomes. SULV, small unilamellar vesicle; LMLV, large multilamellar vesicles. Formulation details are given in Table 1.

obtained from SUVs with those obtained from LMLVs, there were no significant differences between LMLVs and smaller vesicles in all cases, a finding which suggested that the intact vesicles did not permeate through human epidermal membrane in-vitro, based on the assumption that permeation would be a function of carrier diameter. However, reduction of vesicle size improved drug deposition into deeper strata and penetration through skin, with large structures improving deposition only (Valenta & Janisch 2003; Verma et al 2003).

Trotta et al (2002) incorporated an amphiphilic anti-inflammatory drug, dipotassium glycyrrhizinate, into liposomes. They reported that the agent increased the elasticity of the entities. Measuring the vesicle size before and after extrusion through a microporous filter, they concluded that the elastic particles were capable of penetrating a pore with a diameter three times smaller than their own span. However, they were able to show only improved skin deposition of dipotassium glycyrrhizinate. This finding may support the possibility that vesicles can penetrate into but not through skin. In similar studies the size of transfersomes was unchanged after extrusion through semi-permeable membrane barriers. This result was not evident for standard liposomes. In addition, both skin deposition and permeation were measured. The authors reported the presence of the carriers in mice blood after topical application of fluorescent-labelled transfersomes. Noteworthy, the size of these vesicles was similar to that of the starting liposome suspension. This was taken as clear evidence for vesicle invasion into and through skin (Cevc et al 2002; Cevc & Gebauer 2003).

Tape stripping and freeze-fracture electron microscopy indicated fast delivery of intact elastic vesicles into the stratum corneum. This invasion was thought to be via channel-like regions in the stratum corneum. Again, elastic vesicles were superior to rigid nano-aggregates with non-occlusive application being best (Honeywell-Nguyen et al 2003b). In another study, the transport of elastic and rigid vesicle components and a model drug into human skin was monitored in-vivo. A deuterium-labelled phospholipid was incorporated as a marker. It was suggested that elastic vesicle material could rapidly enter the deeper layers of the stratum corneum and could reach almost the stratum corneum-viable epidermal junction. Rigid carrier material did not move deep into the stratum corneum. However, the distribution profile of the drug in the lower stratum corneum layers was different from that of the vesicle material. This suggested that once the elastic vesicles partitioned into the stratum corneum, the drug was released from them (Honeywell-Nguyen et al 2004; Honeywell-Nguyen & Bouwstra 2005).

In light of the above reports, it appears that some vesicles may penetrate intact to some extent into healthy skin. Questions remain as to how deep into the skin strata intact carriers move, and if indeed integral structures can carry their payload through the entire tissue.

Trans appendageal penetration

Occlusive application and full skin hydration is supposedly detrimental for transdermal drug delivery from transfersomes. This effect was attributed to inhibition of the transdermal hydration gradient, which is believed to be the driving force for vesicle-skin penetration (Cevc & Blume 1992; Cevc et al 1995). Another possible explanation is that over-hydration of the skin can swell the corneocytes and thus close or at least minimize the size of shunt routes that may play a role in liposomal skin delivery. This hypothesis will be explored next.

Electron microscopic investigations, employing soya-lecithin, cholesterol liposomes with encapsulated protein G-gold conjugate, indicated that liposomes up to 600-nm diameter could penetrate through skin but those of 1000 nm or more remained interiorized in the stratum corneum. Macrophages accumulated into the corium after vesicle application to rabbit or guinea-pigs. Deposition was higher in hairy guinea-pigs. However, with regard to penetration though skin, no difference could be found between hairless and hairy guinea-pigs. Despite this it was concluded that invasion was mainly along the hair sheath (Schramlova et al 1997). However, these findings can reflect only delivery into, rather than through, the hair follicles. Also, vesicular delivery through shunts was excluded on the basis that there were no significant variations between different animals or man with diverse densities of hair follicles, with regard to the transfersomal input of insulin (Cevc et al 1998).

A novel in-vitro technique using human abdominal skin was developed to explore the role of appendageal transport on liposomal skin delivery of estradiol. The study monitored vesicular delivery through epidermis and compared this with penetration through a sandwich of stratum corneum and epidermis, where the additional stratum corneum formed a top layer. As the orifices of these shunts occupy only approximately 0.1% of the total skin surface area, there was a negligible chance that shunts in the two membranes would superimpose. It was thus assumed that the top layer of stratum corneum would block most of the shunts available in the bottom membrane (El Maghraby et al 2001b). From this study, it was concluded that the shunt routes played a minor role in transdermal delivery of estradiol from liposomes. In a recent study, the transfollicular delivery from liposomes was enhanced only after combination with iontophoresis (Han et al 2004).

In summary, it appears that the shunt routes play no major role in liposomal transdermal delivery. However, vesicle penetration into but not necessarily through hair follicles (i.e. targeting) is clearly demonstrated by numerous literature reports (see above).

Conclusion

Lipid vesicles are promising drug input systems providing both dermal and transdermal delivery. Ultraflexible liposomes usually perform better than traditional vesicles, but for clinical use many problems will need solving with respect to, for example, their stability and scale-up.

The mechanisms of action of liposomes in inserting therapeutic agents into and through human skin remain controversial. 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. Accordingly, it is not possible to describe a general mode or mechanism of action of liposomes acting as skin drug delivery systems and in each situation a detailed description of the formulation and experimental design has to be considered. However, this review suggests that combinations of mechanisms of action probably operate in many situations.

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