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Interactions between liposomes and human stratum corneum studied by freeze-substitution electron microscopy

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Abstract

Vesicular systems such as liposomes have been applied for the percutaneous delivery of drugs. Successful percutaneous delivery relies strongly on an adequate reduction of the barrier properties of the stratum corneum, which is considered to constitute the main barrier of the skin. In this study, the mechanisms by which liposomes interact with the human stratum corneum were investigated using freeze-substitution electron microscopy in combination with ruthenium tetroxide fixation. A liquid-state liposome suspension, containing dilaurylphosphatidylcholine (DLPC) was compared with two gel-state liposome suspensions, containing distearylphosphatidylcholine (DSPC) and ceramides. In addition, the influence of cholesterol sulphate, a component of the skin lipids, was studied. Comparing the liquid-state with the gel-state liposomes, we observed that both gel-state liposome suspensions were able to form large stacks and networks of lipid bilayers at the surface of the stratum corneum in contrast to the liquid-state DLPC liposomes, which showed no adsorption. Deeper down in the stratum corneum inter- and intracellular interactions were observed for the DLPC liposomes, whereas intercellular interactions were observed only for the gel-state liposomes to which cholesterol sulphate was added. Incorporation of cholesterol sulphate in the DLPC-liposomes had no influence on their mechanism of interaction.

Keywords: Stratum corneum; Liposomes; Skin lipids; Ruthenium tetroxide; Electron microscopy; Freeze-substitution

1. Introduction

A major problem in transdermal drug delivery is the low permeability of the most apical layer of the skin, the stratum corneum. The stratum corneum is regarded as a heterogeneous two-com-

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partment system composed of keratin-filled corneocytes, embedded in a lipid-enriched intercellular matrix. This lipid matrix is organised in lamellae (Breathnach et al., 1973; Elias and Friend, 1975) which are formed by rearrangement of lamellar disks that are extruded from the uppermost cells of the stratum granulosum (Lavker, 1979). The permeability barrier is located within the lipid bilayers in the intercellular spaces of the stratum corneum (Elias and Friend, 1975; Wertz and Downing, 1982; Landmann, 1986) and consists of ceramides (40–50%), fatty acids (15–25%), cholesterol (20–25%) and cholesterol sulphate (5–10%) (Gray et al., 1982; Wertz and Downing, 1983; Long et al., 1985; Wertz et al., 1985; Melnik et al., 1989). In order to increase the permeation of drugs, several approaches have been developed that should enable drugs to penetrate the stratum corneum, both intercellularly through the lipid bilayers as well as transcellularly through the corneocytes, e.g. coapplication of penetration enhancers and iontopheresis (Craane-van Hinsberg et al., 1994). However, the use of penetration enhancers may be limited by their toxicity (Imokawa et al., 1975; Bouwstra et al., 1992, 1996; Wilhelm et al., 1993). In addition, research has been carried out into the field of vesicular transport systems, e.g. liposomes and non-ionic vesicles (niosomes) for transdermal drug delivery (Lasch and Wohlrab, 1986; Bouwstra et al., 1991a; Lasch et al., 1991; Hofland, 1992; van Hal, 1994; Korting et al., 1995; Cevc et al., 1996; Kirjavainen et al., 1996; Schaller et al., 1997). Their ability to change the drug disposition in the body is a possible advantage for their use as drug carrier systems.

There are several possible mechanisms proposed, depending on the composition of the vesicles, that are responsible for an increased drug transport across the skin. First, it might be possible that the vesicles penetrate the stratum corneum intact (Mezei and Gusalekharam, 1980; Lasch and Wohlrab, 1986; Cevc and Blume, 1992), secondly, lipid bilayer stacks are formed on the surface of the stratum corneum (Ganesan et al., 1984; Lasch and Wohlrab, 1986; Weiner et al., 1989; Hofland, 1992), thirdly, vesicle fragments penetrate into the stratum corneum (Lasch and Wohlrab, 1986; Weiner et al., 1989; Boddé et al., 1992; Hofland, 1992; van Hal, 1994) or, finally, the vesicles do not interact with the stratum corneum.

The goal of this study was to investigate the interactions between human stratum corneum and liquid-state liposomes, gel-state liposomes or liposomes that have a composition similar to that of the stratum corneum lipids. The interactions were examined by using freeze-substitution electron microscopy in combination with ruthenium tetroxide (RuO_4) fixation, which provides more insight into their mode of action and transport pathways through the skin.

In addition, the influence of cholesterol sulphate as a constituent was studied, since it is believed that cholesteryl sulphate plays a major role in the desquamation process of the corneocytes. When the cholesterol sulphate content in the stratum corneum is changed, an impaired desquamation process is observed (Williams and Elias, 1981).

2. Materials and methods

2.1. Chemicals

Bovine brain ceramide 3 and cholesteryl-3-sulphate were obtained from Sigma Chemicals (St. Louis, MO). Distearylphosphatidylcholine (DSPC) and dilaurylphosphatidylcholine (DLPC) were a kind gift from Rhone-Poulenc-Natterman.

2.2. Stratum corneum preparation

Fresh human abdomen or mamma skin was obtained after cosmetic surgery and processed the same day. After removal of the subcutaneous fat, the skin was dermatomed to 250 μm (Padgett Dermatome, Kansas City, KS) and incubated overnight on filter paper soaked in a 0.1% (w/v) trypsin solution (Type 3, Sigma, St. Louis, MO) at 4°C. The skin was then incubated for 1 h at 37°C. The stratum corneum was physically removed from the epidermis, washed with a 0.1%

(w/v) anti-trypsin solution (Sigma) to block enzyme activity and washed twice with distilled water. The stratum corneum was dried and stored in a desiccator over silica gel under nitrogen at room temperature. Before use, the stratum corneum was rehydrated by placing it carefully in a petri dish with PBS, pH 7.4.

2.3. Preparation of liposome suspensions

The liposome suspensions were prepared by a modification of the film method described by Baillie et al. (1985). The lipids were dissolved in a 3:1 (v/v) mixture of chloroform and methanol. After evaporation of the solvent under vacuum overnight, the lipid films were hydrated above the gel–liquid transition temperature ($T_c = 53°C$) of the lipids using PBS, pH 7.4. During hydration the suspensions were vortexed.

Subsequently, the suspensions were sonicated for 5 min using a Branson Sonifier 250 (Branson, Danbury, CT), with an 1/8-inch microtip at 30 W energy output.

Sample volumes of 5 ml were prepared with a final lipid concentration of 5% (w/v). The liposomes consist of either 48% (v/w) lipids, 30% (v/w) cholesterol and 22% (v/w) fatty acid or 45.6% (v/w) lipids, 28.5% (v/w) cholesterol, 20.9% (v/w) fatty acid and 5.0% (v/w) cholesterol sulphate (see Table 1).

Table 1
Composition of the liquid- and gel-state liposome suspensions

Liposome composition	Ratio (%, w/w)
Liquid-state vesicles	
DLPC:cholesterol:stearic acid	48.0:30.0:22.0
DLPC:cholesterol:stearic acid:cholesterol sulphate	45.6:28.5:20.9:5.0
Gel-state vesicles	
DSPC:cholesterol:stearic acid	48.0:30.0:22.0
DSPC:cholesterol:stearic acid:cholesterol sulphate	45.6:28.5:20.9:5.0
CER:cholesterol:stearic acid	48.0:30.0:22.0
CER:cholesterol:stearic acid:cholesterol sulphate	45.6:28.5:20.9:5.0

2.4. Dynamic light scattering (DLS)

The z-average diameters of the liposomes were determined by Dynamic Light Scattering (DLS) using a Malvern 4700 (Malvern Ltd., Malvern, UK) with a 25-mW He-Ne laser and the Automeasure version 3.2 software immediately after preparation and 24 and 48 h after preparation. For viscosity and refractive index the values of pure water were used. The samples were diluted in order to avoid multiple scattering. The polydispersity index, which is a measure of the homogeneity of a sample, ranges from 0.0 (monodisperse) to 1.0 (very heterogeneous). All formulations were prepared and measured in triplicate.

2.5. Incubation skin and liposomes

The stratum corneum incubation experiments were carried out under occlusion in closed Teflon diffusion cells. Stratum corneum samples (Ø16 mm) were supported by a Silastic membrane (Dow Corning 500-1, non-sterile, non-reinforced) and clamped between the donor and acceptor compartment with the stratum corneum facing the donor compartment. The liposome suspensions (200 μl) were administered into the donor compartment for 24 h at room temperature. The acceptor compartment contained PBS. The control sample was treated with PBS.

After the 24-h incubation period the stratum corneum was removed and prepared for fixation. All liposomal suspensions were administered to at least three different donor skin samples.

2.6. Fixation of the skin

The stratum corneum was cut into small ribbons with a size of approximately 2×1 mm. The ribbons were fixed in 5% (w/v) glutaraldehyde (GA) in 0.1 M sodium cacodylate buffer, pH 7.2, and postfixed in 0.2% (w/v) ruthenium tetroxide (RuO_4) in sodium cacodylate buffer, pH 6.8, with 0.25% (w/v) potassium ferrocyanide ($K_4Fe(CN)_6$). After 1 h, the RuO_4 solution was replaced by fresh RuO_4 in order to establish an optimal fixation. After rinsing in buffer, the tissue samples

were cryofixed, by rapid freezing on a liquid nitrogen-cooled metal mirror (KF80, Reichert-Jung, Austria) at −196°C. The samples were stored in liquid nitrogen at −196°C prior to freeze-substitution.

2.7. Freeze-substitution

The freeze-substitution procedure was carried out in a CS-Auto (Reichert-Jung). The tissue samples were dehydrated at −90°C for 48 h using 100% methanol, containing 1.0% (w/v) osmium tetroxide (OsO_4), 0.5% (w/v) uranyl acetate and 3.0% (w/v) GA (Müller et al., 1980; Humbel et al., 1983). After the 48-h substitution period, the temperature was raised to −45°C, the samples were washed 3 times in 100% methanol, and subsequently the methanol solution was gradually replaced by the embedding medium, Lowicryl HM20 (Carlemalm et al., 1982).

The Lowicryl HM20 (100%) was replaced after 24 and 48 h by freshly made embedding medium. Finally the samples were transferred to a mould, containing Lowicryl HM20, and were incubated for 48 h at −45°C under UVA-radiation, to allow polymerisation.

Ultrathin sections were cut (Ultracut E, Reichert-Jung), transferred to formvar-coated grids and examined in a 201EM electron microscope (Philips, Eindhoven, The Netherlands). For each sample, 10 overview and approximately 30–40 detail electron micrographs were taken.

3. Results

3.1. Dynamic light scattering

The average size of the liposomes, measured by dynamic light scattering, ranged between 120 and 200 nm, whereas the polydispersity ranged from 0.15 to 0.30.

3.2. Ultrastructure of normal untreated human stratum corneum

3.2.1. Intercellular ultrastructure

Micrographs of untreated stratum corneum are shown in Figs. 1 and 2. Fig. 1 shows an example of the limited diffusion of RuO_4 into the stratum corneum samples. The appearance of the stratum corneum did not differ significantly between the various individuals and between mamma and abdomen skin. The lipid bilayers within the intercellular domains of PBS-treated stratum corneum exhibit a similar organisation and structure as described previously (Madison et al., 1987; Swartzendruber et al., 1987), and are mainly organised in an alternating pattern of electron-dense and lucent bands, the so-called Landmann units. However, due to the poor penetration of the ruthenium tetroxide RuO_4, the lipid bilayers are not consistently visualised in the tissue samples; the upper and lowest cell layers are fixed in contrast to the cell layers in the centre of the stratum corneum (Fig. 1).

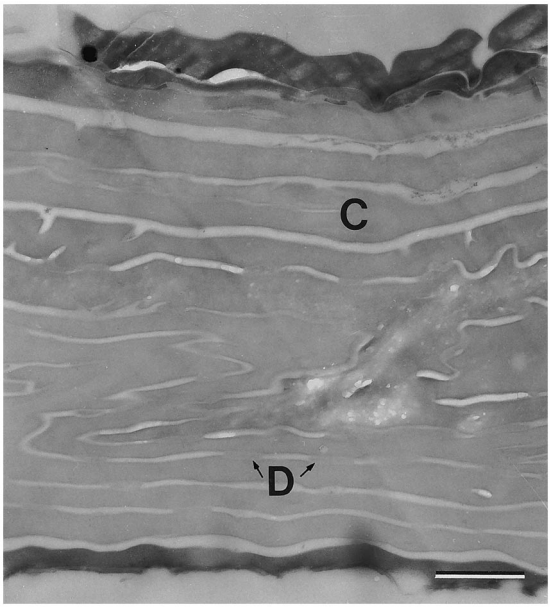

Fig. 1. Overview of the stratum corneum. C, corneocyte, D, desmosome. Bar = 1 μm.

The number of Landmann units throughout the specimen differs from one cell layer to another, and even within the same intercellular space disordering or dislocation of the Landmann units can occur. Fig. 2a and Fig. 2e show examples of such dislocations.

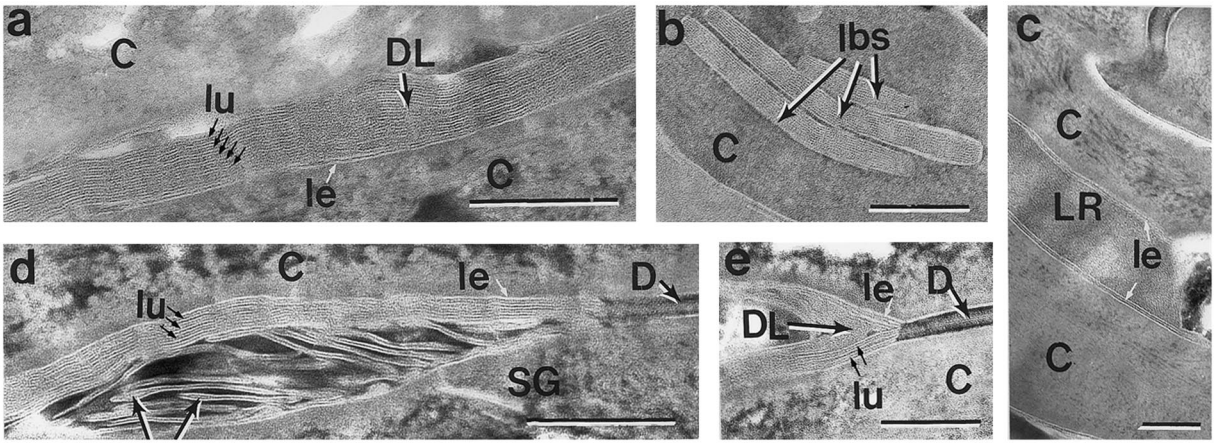

Fig. 2. Detailed micrographs of control stratum corneum. (a) Lipid bilayers organized in Landmann unit pattern. (b) Rectangular lipid bilayer structures, intracellular. (c) Lipid regions, intercellular. (d) Lamellar body extrusion at stratum corneum–stratum granulosum interface. (e) Dislocation in intercellular lipid bilayers. C, corneocyte; DL, dislocation; arrowhead, electron-lucent band in lamellar disk; le, lipid envelope; D, desmosome; lu, Landmann unit; LR, lipid region; SG, stratum granulosum; lbs, lipid bilayer structures. Bar = 200 nm.

In the intercellular space of the upper cell layers of the stratum corneum, large areas of grey regions were visualised (Fig. 2c), whereas only small grey regions were present in the lower parts of the stratum corneum. The lipid envelope is still present at the boundaries adjacent to these non-lamellar regions (Fig. 2c). The origin of these regions is unknown and has not been reported before. However, upon hexane extraction these regions have disappeared. Since hexane is known for its ability to extract triglycerides, this observation suggests that these non-lamellar regions might consist mainly of triglycerides originating from, for example, the sebum (unpublished observations), or from contamination of the underlying fat regions during the surgical removal of the skin.

3.2.2. Intracellular ultrastructure

The corneocytes show a regular keratin pattern. Rectangular structures containing lipid lamellae were occasionally observed (Fig. 2b). These lipid lamellae showed a pattern of electron-lucent and -dense bands, characterised by an approximately equal width and the distance between these lamellae is estimated smaller than those observed in the intercellular space (Madison et al., 1987). The origin of these rectangular structures is not well understood and has not been described in detail before. They might be explained as lamellar body remnants; however, because of their rectangular shape and the small repetition distance this seems unlikely.

3.2.3. Lamellar body extrusion

After separation of the stratum corneum from the epidermis, parts of the stratum granulosum were still attached to the stratum corneum in which the lamellar body extrusion process could be visualised. Fig. 2d shows the lamellar body extrusion process at the interface of stratum granulosum and stratum corneum. The lamellar bodies extrude lamellar disk-like structures into the intercellular space, where they fuse edge-to-edge to form the lipid bilayers. Occasionally, an electron-lucent region was observed within a lamellar disk (Fig. 2d, arrow) and, furthermore, the electron-lucent pattern was changed after extrusion of the lamellar disks into the intercellular space. An electron-lucent band appears to be added in between the broad lucent bands originating from the lamellar disks during the formation of the lipid bilayers.

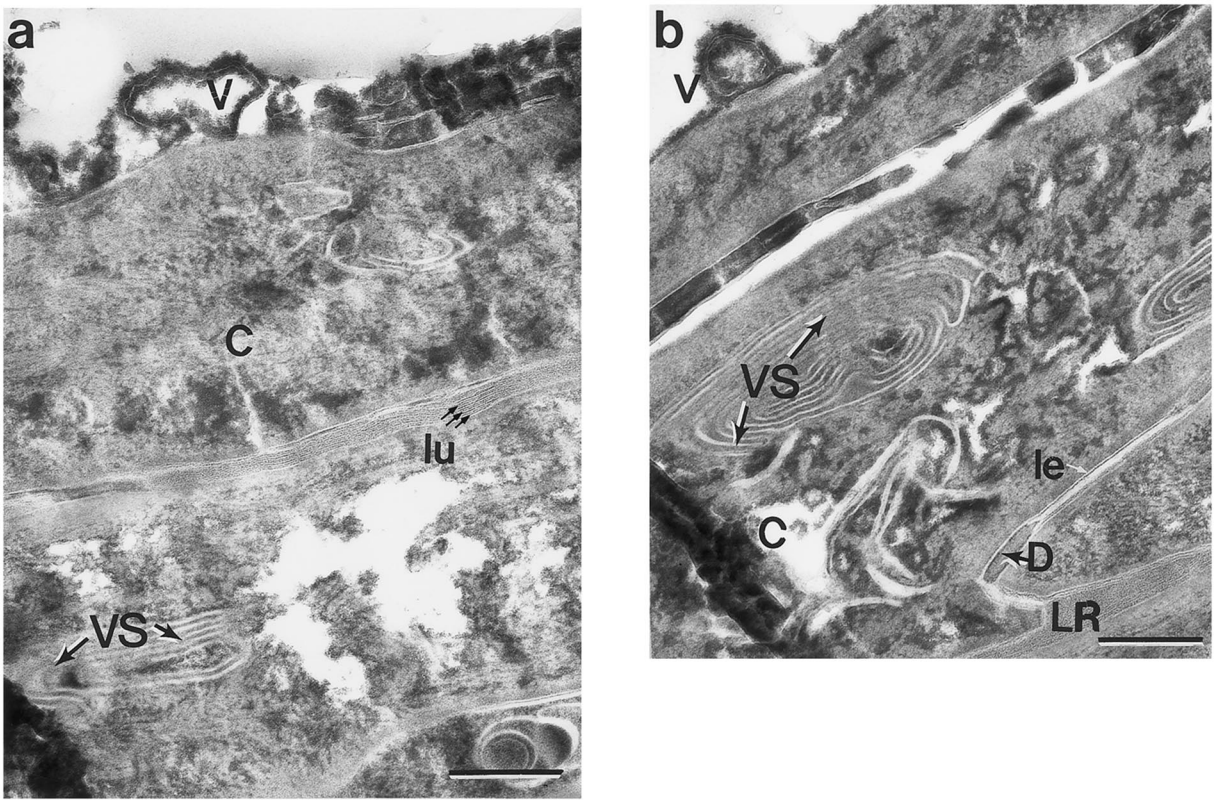

Fig. 3. DLPC–liposome interaction: (a) without and (b) with cholesterol sulphate. V, vesicle; lu, Landmann unit; C, corneocyte; VS, vesicle structure; le, lipid envelope; LR, lipid region. Bar = 200 nm.

3.2.4. Desmosomes

Desmosomes were observed at irregular intervals throughout the specimen (Fig. 2c–e). In the lower parts of the stratum corneum the desmosomes appear as electron-dense longitudinal structures, whereas in the upper parts of the stratum corneum, where the desmosomes are degraded, they appear as more 'lens'-like structures (Hou et al., 1991) partly or completely surrounded by lipid bilayers.

3.3. Interactions between liposomes and stratum corneum

3.3.1. Liquid-state liposomes

3.3.1.1. DLPC-liposomes. The ultrastructure of the stratum corneum after treatment with the liquid-state DLPC liposomes without and with cholesterol sulphate are shown in Fig. 3a and Fig. 3b, respectively.

DLPC-liposomes could be visualised on the surface of the stratum corneum (Fig. 3). No fusion or lamellar stack formation was observed on the surface of the stratum corneum. Furthermore, no major changes were observed in the intercellular lipid lamellar organisation. However, vesicular structures were visualised within the upper corneocytes after treatment with both DLPC liquid-state liposome suspensions. The distance between the lipid bilayers of these vesicular structures is significantly broader than that of the intercellular lipid bilayers, lamellar bodies and multilamellar vesicles, which is approximately 6 nm.

There is no significant difference in ultrastructure upon addition of cholesterol sulphate to the DLPC-liposomes.

3.3.2. Gel-state liposomes

3.3.2.1. DSPC-liposomes. Both DSPC-liposome suspensions formed networks and stacks of lipid bilayers at the interface of the suspension and the stratum corneum (Fig. 4a, Fig. 4b). No changes were observed in the intercellular lipid organisation, except for the DSPC-liposomes with cholesterol sulphate shown in Fig. 4b where, occasionally, in the upper cell layers, lipid bilayers were disrupted.

3.3.2.2. Ceramide-liposomes. Stacks and networks of lipid bilayers were formed by both the ceramide-liposome suspensions at the stratum corneum–suspension interface (Fig. 5a, Fig. 5b).

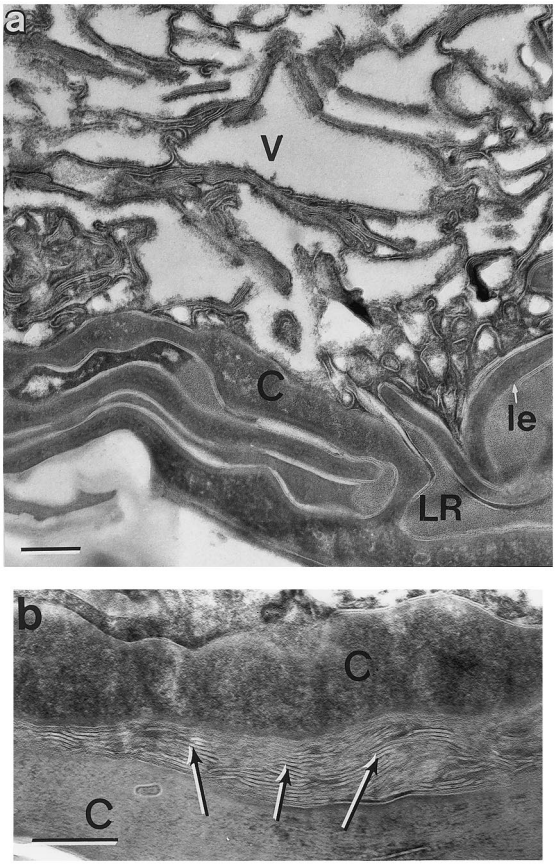

Fig. 4. DSPC–liposome interaction: (a) without and (b) with cholesterol sulphate. V, vesicle; C, corneocyte; le, lipid envelope; LR, lipid region. Bar = 200 nm.

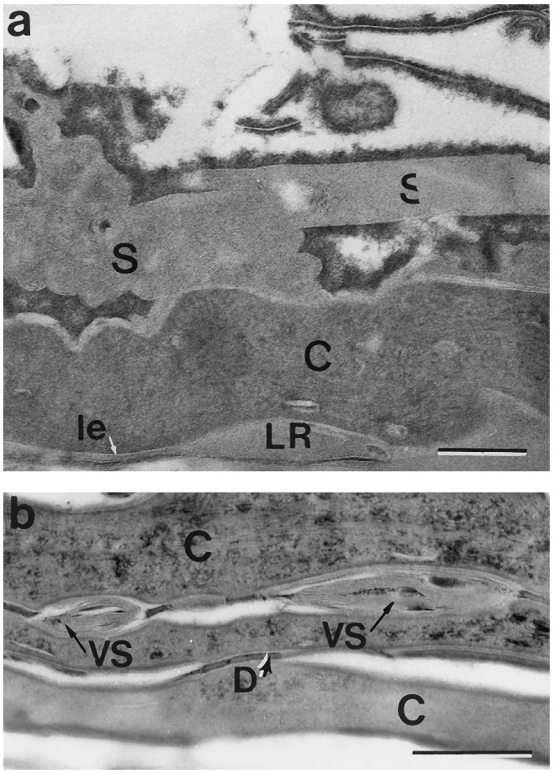

Fig. 5. Ceramide–liposome interaction: (a) without and (b) with cholesterol sulphate. C, corneocyte; le, lipid envelope; LR, lipid region; S, stack of lipid bilayers; VS, vesicular structure; D, desmosome. Bar = 200 nm.

No structural changes were observed in the stratum corneum after treatment with the ceramide-liposomes prepared without cholesterol sulphate (Fig. 5a). The ceramide-liposome suspension prepared with cholesterol sulphate showed intercellular vesicular structures, as shown in Fig. 5b, in the upper parts of the stratum corneum.

The results of the treatment of stratum corneum with the liposome suspensions are summarised in Table 2.

4. Discussion

Although there is a general agreement that liposomes as drug carriers enhance the penetration of drugs through the skin, little is known about the actual mechanisms of interactions be-

Table 2
Liposomes interactions with human stratum corneum

Liposome suspension	Physical state	Adsorption	Structural changes	
			Intercellular	Intracellular
DLPC	Liquid	−	+	+
DLPC[a]	Liquid	−	+	+
DSPC	Gel	+	−	−
DSPC[a]	Gel	+	±	−
Ceramides	Gel	+	−	−
Ceramides[a]	Gel	+	+	−

Summary of the interactions between the liposome suspensions and the stratum corneum. Both the adsorption of the liposomes at the surface of the stratum corneum and the structural interactions were investigated.
[a] Liposomes prepared with cholesterol sulphate.
+, frequently observed; ±, occasionally observed; −, not observed.

tween vesicles and the stratum corneum. Freeze-fracture electron microscopy (FFEM) and confocal laser scanning microscopy (CLSM) studies have been performed involving the investigation of vesicle–skin interactions (Hofland, 1992; van Hal, 1994; Kirjavainen et al., 1996). Korting et al. (1995) and Schaller et al. (1997) have used transmission electron microscopy in combination with osmium tetroxide to study the interactions of liposomes with human epidermis reconstructed in vitro. In this study, however, transmission electron microscopy in combination with ruthenium tetroxide and freeze-substitution was used, since it offered us the possibility to fix the lipid lamellae and to examine individual lamellae in cross-sections of the stratum corneum. In addition, we compared ceramide gel-state vesicles, with a composition similar to the skin lipids, with gel-state and liquid-state liposomes containing phospholipids. Recently, we performed studies on the phase behaviour ceramides, fatty acids, cholesterol sulfate and cholesterol (Bouwstra et al., 1991b). These studies revealed that cholesterol sulfate is solubilized in the lamellar phases. Since the relative amounts of the various classes of lipids in these lamellar phases are similar to that used in the preparation of the liposomes, it is very likely that cholesterol sulphate is distributed homogeneously in the liposomal bilayers.

4.1. Untreated stratum corneum

In the electron micrographs of untreated stratum corneum, various interesting features were observed. Fig. 2d shows the extrusion process during which the lamellar bodies extrude lamellar disks into the intercellular space at the stratum granulosum–stratum corneum interface. If the lamellar disks fuse edge-to-edge, without any change in lipid organisation within the disks, the Landmann unit pattern with a broad–narrow–broad electron-lucent pattern would not be formed (Fig. 2a). From this observation we conclude that a change in electron-lucent band pattern occurs and that this change might be due to either a rearrangement of the lipids during the fusion process or to a fixation artefact. Most probably during the fusion process glycosylceramides are partly transformed into ceramides. It is likely that, due to this change in lipid composition, also a change in long-range ordering occurs inducing the formation of Landmann units.

In the intercellular space of the upper cell layers of the stratum corneum, large areas of non-lamellar electron-dense material were visualised (Fig. 2c), whereas only small non-lamellar regions were present in the lower parts of the stratum corneum. Adjacent to these non-lamellar regions, the lipid envelope is still present at the cell boundaries (Fig. 2c). The origin of these non-lamellar regions is unknown and has not been reported before.

However, upon hexane extraction these regions disappeared (unpublished observations). Since hexane is known for its ability to extract triglycerides, this observation suggests that these non-lamellar regions might consist of triglycerides originating from, for example, the sebum, that diffuse in between the desquamating upper cell layers or from contamination of subcutaneous fat regions during the surgical removal of the skin. Occasionally, a few lipid bilayers were still present within these non-lamellar electron-dense regions.

In order to investigate the function of the lipid envelope we performed additional heating experiments in which the stratum corneum was heated to 120°C, at which the constituents of the lipid bilayers are in an isotropic liquid state (unpublished results). Subsequently the samples were cooled down to room temperature in order to recrystallize the lipids. In former experiments, in which the stratum corneum was treated in the same manner, thin layer chromatography showed no degradation of the lipids during the heating–cooling cycle of the stratum corneum (Bouwstra et al., 1991b). Fig. 6 shows the ultrastructure of the stratum corneum after lipid recrystallisation. The lipid envelope is still intact adjacent to the cell membrane. Furthermore, in those areas in which bilayers were visualised, these bilayers were organised in the Landmann unit pattern. In contrast to the untreated control stratum corneum; however, the lipid bilayers were not oriented parallel to the lipid envelope (Fig. 6, inset). This observation strongly indicates that the lipid envelope itself is not a prerequisite for the actual formation of the lipid bilayers, but that it serves as a template for the formation of the lipid bilayers at the stratum corneum–stratum granulosum interface, and thus dictates the orientation of the lamellae. The template results in the parallel orientation of the lipid bilayers to the lipid envelope and suggests that the lipid envelope is important for the barrier function of the stratum corneum, since a random disordered distribution of the lamellae in the intercellular spaces would enable molecules to diffuse freely through the stratum corneum, as is observed upon treatment with penetration enhancers (e.g. azones) which disturb the lamellae organisation (Bouwstra et al., 1992, 1996, 1998).

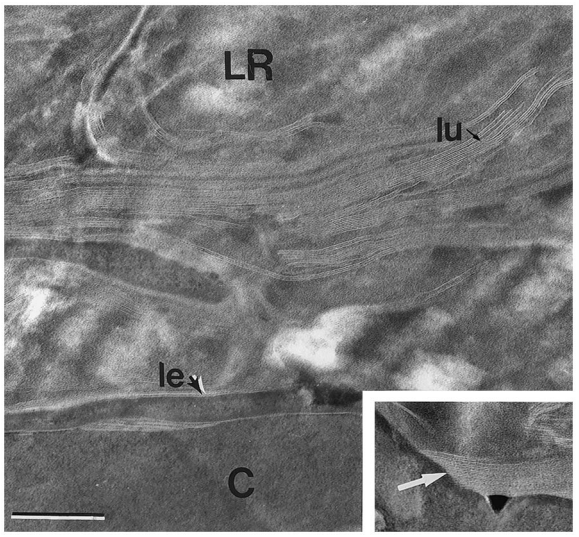

Fig. 6. Ultrastructure human stratum corneum after heating to 120°C and subsequent cooling down to room temperature. C, corneocyte; le, lipid envelope; LR, lipid region; lu, Landmann unit. Bar = 200 nm. Inset: arrow, lipid bilayers, oriented perpendicular to the cell membrane.

4.2. Liposome–stratum corneum interactions

In spite of the similar composition of the ceramide liposomes and the stratum corneum lipid bilayers, the ceramide liposomes do not produce the Landmann unit bilayer pattern when fusing at the stratum corneum interface (Fig. 5b). It seems that the bilayers prepared with bovine brain ceramide 3 cannot form the characteristic Landmann units upon fusion, in contrast to liposomes, prepared from isolated ceramides (Abraham and Downing, 1990).

The gel-state liposomes, composed of ceramides and DSPC, both with and without cholesterol sulphate, aggregate, fuse and adhere on the stratum corneum surface, thereby depositing stacks of lamellar sheets and forming lipid bilayer networks (Fig. 4a,b). It is possible that vesicle aggregation and fusion are induced by either the presence of corneocytes or the lipid lamellae or by the lower pH of the stratum corneum (pH 5–6), since the vesicles would not fuse in suspension. The less-rigid DLPC liquid-state liposomes, however, are not able to aggregate and fuse on the surface of the stratum corneum. From this observation we

conclude that, in order to establish networks and stacks on the stratum corneum surface, it is necessary that the vesicles exist in the gel-state rather than in the liquid state.

The presence of intracellular vesicular structures was rarely observed in stratum corneum incubated with PBS or after incubation with gel-state liposomes, with and without cholesterol sulphate. Occasionally, intracellular vesicular structures were observed after treatment with DLPC liquid-state liposomes, both with and without cholesterol sulphate. These vesicular structures were observed only in the superficial layers of the stratum corneum, and their appearance might be explained by desquamating corneocytes with a leaky membrane, through which the liquid-state liposomes penetrate. As a result of their fluidity, the liquid-state vesicles are more flexible, and therefore deform more easily compared to the more rigid gel-state liposomes.

The electron micrographs also depict ultrastructural changes in the intercellular lipid regions of the stratum corneum after incubation with DSPC and ceramide gel-state liposomes, both with cholesterol sulphate. These changes were not found after incubation with PBS, DSPC or ceramide gel-state liposomes, both without cholesterol sulphate. After incubation with cholesterol sulphate containing ceramide gel-state liposomes vesicular structures were visualised in between the upper and lower corneocytes (Fig. 5b), whereas after incubation with cholesterol sulphate containing DSPC gel-state liposomes the cohesion between lipid bilayers in the upper cell layers decreased occasionally. There are at least two explanations possible for the presence of intercellular vesicular structures after the application of the ceramide liposomes. First, ceramide liposomes migrate through the intercellular lipid bilayers of the stratum corneum. However, this seems very unlikely since the average size of the ceramide gel-state liposomes, with cholesterol sulphate, measured by dynamic light scattering, was approximately 150 nm. This size is very large compared to the distance between the intercellular lipid bilayers, the average size of one Landmann unit being ± 12.8 nm (Madison et al., 1987). Secondly, liposomal constituents are able to mix and diffuse by molecular dispersion through the intercellular lipids, inducing ultrastructural changes in some regions. The cholesterol sulphate may be responsible for the fusion of the gel-state liposomes with the intercellular lipid bilayers and reduce the cohesion between the lipid lamellae. If the cohesion is reduced or lost, the appearance of vesicular structures between the corneocytes may then be explained by migration of vesicles between the corneocytes.

The reduced cohesion induced by cholesterol sulphate would also explain the ultrastructural changes observed in between the upper cell layers of the stratum corneum, after incubation with DSPC gel-state liposomes. However, no vesicular structures were formed but, instead, a disruption of the intercellular lipid bilayers was observed. It can be concluded, when comparing DSPC and ceramide gel-state liposomes, that the addition of cholesterol sulphate, changed the interactions between the gel-state liposomes and the stratum corneum.

In summary, we did not observe intact liquid- or gel-state liposomes, applied occlusively, in the lower regions of the stratum corneum. Occasionally liposomes caused changes in the lipid bilayer structure in the stratum corneum. Vesicular structures are mainly observed in the upper cell layers of the stratum corneum, probably due to the impaired barrier function as a result of the desquamation process of the corneocytes.

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