Transdermal Delivery of Pergolide from Surfactant-Based Elastic and Rigid Vesicles: Characterization and *in Vitro* Transport Studies

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Received February 28, 2002; accepted April 1, 2002

Purpose. The aim of this study was to investigate the effect of elastic and rigid vesicles on the penetration of pergolide across human skin.
Methods. Vesicles used consisted of the bilayer-forming surfactant L-595 (sucrose laurate ester) and the micelle-forming surfactant PEG-8-L (octaoxyethylene laurate ester), together with the stabilizer sulfosuccinate. A series of L-595/PEG-8-L/sulfosuccinate vesicles were investigated, ranging from very rigid to very elastic. Pergolide-loaded elastic and rigid vesicles were visualized using Cryo-TEM and characterized for size and stability. Transdermal penetration of pergolide from different vesicle compositions was studied *in vitro* using flow-through Franz diffusion cells. A saturated buffer solution served as the control.
Results. Vesicle composition had a major effect on the physicochemical characteristics, morphology and drug solubility of the vesicular system. L-595/PEG-8-L/sulfosuccinate (70/30/5) elastic vesicles gave the best balance between vesicle stability and elasticity, as well as the highest drug solubility. Transport studies clearly showed that elastic vesicles were superior to rigid vesicles. Elastic vesicles enhanced the drug transport compared to the buffer control, although rigid vesicles decreased the drug transport. The best drug transport was achieved from L-595/PEG-8-L/sulfosuccinate (70/30/5) elastic vesicles, resulting in a steady-state flux of 13.6 ± 2.3 ng/(h*cm^2). This was a 6.2-fold increase compared to the most rigid vesicles.
Conclusions. This study supports the hypothesis that elastic vesicles are superior to rigid vesicles as vehicles for transdermal drug delivery.

KEY WORDS: transdermal delivery; elastic vesicles; pergolide.

INTRODUCTION

Transdermal drug delivery has many advantages compared to other routes of drug administration. However, a major problem still remains the poor penetration of most compounds across human skin. Vesicular systems were introduced in the early 80s and have since been studied as vehicles for dermal and transdermal drug delivery. Many reports have shown enhanced skin permeation when drugs were incorporated into vesicles (1–3). Yet, the exact mechanism by which drug transport is promoted, is still not fully understood. It has been suggested that this mechanism and thus also the enhancement effect, is strongly dependent on the physicochemical characteristics of the vesicular systems (4–6). Liquid-state vesicles were found to have superior characteristics to gel-state vesicles for enhancement of skin permeability (5). In the present study, liquid-state elastic vesicles are compared to liquid-state rigid vesicles.

Elastic vesicles are one of the most promising developments in vesicular systems design. They have been suggested to be more effective than conventional rigid vesicles as a vehicle for transdermal drug delivery. Elastic vesicles were introduced by Cevc *et al.* (7), who combined phosphatidylcholine with the edge-activator sodium cholate to form flexible membranes. These vesicles, also called Transfersomes, were therefore characterized by their high elasticity. It has been suggested that these Transfersomes were more effective than standard liposomes in the transport of insulin and lidocaine across mouse and human skin (8,9). Using the same vesicle components, El Maghraby *et al.* found that elastic vesicles greatly improved the transport of oestradiol across human cadaver skin compared to a saturated aqueous solution (10). They also introduced Span 80 and Tween 80 as alternative edge-activators that were as effective as sodium cholate. In two other recent studies, lecithin was combined with sodium cholate to form elastic vesicles. These proved to be better than conventional rigid vesicles in enhancing the transport of cyclosporin A and insulin across mouse skin (11,12).

In 1998 Van den Bergh *et al.* introduced a new series of elastic vesicles, consisting of the bilayer-forming surfactant L-595 (sucrose laurate ester) and the micelle-forming surfactant PEG-8-L (octaoxyethylene laurate ester) (13). Incorporation of a micelle-forming component into a vesicle bilayer would result in solubilization of the bilayer and thereby increasing the elasticity of the vesicular system. Therefore, by changing the ratios of the bilayer forming and the micelle-forming surfactants, a series of vesicles can be obtained, ranging from very rigid to very elastic. Although vesicle elasticity could improve drug transport, it should be noted that increasing vesicle elasticity would result in decreasing vesicle stability. Hence, for an efficient drug delivery system, an optimal balance is needed at which vesicles are most elastic, yet still stable.

L-595/PEG-8-L elastic and rigid vesicles were characterized for size and stability in previous studies (13). Vesicle elasticity/rigidity was thoroughly measured using extrusion and the visualization by electron microscopy of pore-membranes after extrusion (14). These studies confirmed that vesicle elasticity increased with increasing molar contents of the micelle-forming component PEG-8-L. Furthermore, electron spin resonance studies demonstrated a systematic increase in the mobility of surfactants at increasing molar contents of PEG-8-L. This might be related to the increase in elasticity. Subsequent studies have shown that L-595/PEG-8-L elastic vesicles could alter the structure of human stratum corneum *in vitro* (6) and hairless mouse skin *in vivo* (15). It has also been found that these elastic vesicles were more effective than rigid vesicles in enhancing the penetration of ^3H_2O across hairless mouse skin (15).

Previous characterization and transport studies using

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L-595/PEG-8-L vesicles, were performed with "empty" vesicles. However, drug molecules could be incorporated into the vesicle bilayers, which could potentially result in an effective transdermal drug delivery system. In the present study, the model-drug pergolide was dissolved into elastic and rigid L-595/PEG-8-L vesicle formulations. Pergolide is a dopamine agonist used in the treatment of Parkinson's disease (16). It is a small lipophilic compound with a MW of 314.5 and a log P of 2.3. To date, little is known about the transport of pergolide across animal or human skin. However, a transdermal drug delivery system for Parkinson's disease would greatly improve its management. Transdermal drug administration could provide a constant drug input. This could significantly reduce the side effects of anti-Parkinson's treatment, which are thought to be due to erratic drug plasma levels. Pergolide is a suitable candidate for transdermal drug delivery due to its potency and therefore only requiring low therapeutic levels. Furthermore, it undergoes extensive first-pass metabolism, which will result in a low bioavailability and a wide intersubject variation of plasma levels after oral administration. Both problems could be overcome by an effective transdermal drug delivery system.

The aim of our study was to characterize pergolide-loaded vesicles and to investigate the effect of elastic and rigid vesicles on the penetration of pergolide across human skin. For this purpose, several L-595/PEG-8-L vesicle compositions were investigated, ranging from very rigid to very elastic. This enabled us to establish an optimal balance between vesicle stability, elasticity and effectiveness as a skin delivery vehicle.

MATERIALS AND METHODS

Materials

L-595 consisted of 100% sucrose laurate ester (30% mono-, 40% di-, and 30% tri-ester) and was kindly supplied by Mitsubishi Kasei (Tokyo, Japan). The octaoxyethylene laurate ester (PEG-8-L) was a gift from Lipo Chemicals (Paterson, New Jersey) and the sodium sulfosuccinate was a gift from Cytec (Rotterdam, The Netherlands). Pergolide was very kindly provided by Eli Lily (Greenwich, Indiana). All other chemicals used were of analytical grade.

Methods

Preparation of Pergolide-Loaded Vesicle and Micelle Solutions

Elastic vesicles consisted of L-595 and PEG-8-L in different molar ratios. Rigid vesicles only contained L-595. As comparison, PEG-8-L micelle solutions were also investigated. The composition and molar ratio of the vesicle and micelle formulations are shown in Table I. Vesicle elasticity was increased by added increasing amounts of PEG-8-L. Vesicles were considered to be elastic when it was possible to extrude the formulations through membranes with pore sizes of 30nm. Rigid vesicles could not be extruded through these membranes. A small amount of sulfosuccinate was added to elastic and rigid vesicles to increase vesicle stability. However, for clarity, all vesicle compositions will be indicated later only by the molar ratio between the two main components L-595 and PEG-8-L, whose molar ratios were systematically changed during the study. The L-595 and PEG-8-L content will be expressed as mol% of the total moles of L-595 and PEG-8-L only.

Vesicles were prepared by modification of the sonication method as described by Baillie *et al.* (17). Briefly, the surfactants and pergolide were dissolved in ethanol, while sulfosuccinate was dissolved in an ethanol/isopropanol mixture. The solutions were added together in appropriate ratios. The organic solvents were then evaporated overnight in a vacuum centrifuge and the remaining lipid film was rehydrated with a 0.05M citrate buffer solution at pH 5.0. Subsequently, vesicle solutions were sonicated using a Branson Sonifier 250 (Branson Ultrasonics Corp., Danbury, United Kingdom) with a 1/8 inch microtip at 60 Watt energy output. The micelle solutions were prepared as above, but without further sonication. All final vesicle and micelle formulations contained 10% w/w of lipids and pergolide at saturated concentrations.

Solubilization of Pergolide into Vesicle and Micelle Solutions

Pergolide was dissolved into vesicle and micelle solutions at saturated concentrations to obtain equal and maximum thermodynamic activities. To determine the maximum amount of drug that could be dissolved, increasing amounts of pergolide were added during preparation of vesicle and micelle solutions. It was assumed that the presence of pergolide crystals would indicate that the solution was saturated with pergolide. Therefore, all pergolide-loaded vesicle and micelle solutions were examined for crystals over a period of 14 days using light microscopy.

Size and Stability Studies

The z-average diameter and polydispersity indexes of all vesicle formulations were measured by Dynamic Light Scattering (DLS) using a Malvern 4700 (Malvern Ltd., Malvern,

Table I. Composition of Vesicle or Micelle Solutions and Their Rigidity/Elasticity

Formulation	Molar ratio L-595/PEG-8-L/sulfosuccinate	Systematic change in composition	Rigidity/elasticity[b]
L-595/PEG-8-L/sulfosuccinate[a]	100/0/10		Most rigid
L-595/PEG-8-L/sulfosuccinate	90/10/10		Rigid
L-595/PEG-8-L/sulfosuccinate	70/30/5		Elastic
L-595/PEG-8-L/sulfosuccinate	50/50/5	Increasing PEG-8-L	Most elastic
L-595/PEG-8-L/sulfosuccinate	0/100/0	Increasing elasticity	Micelles

[a] L-595 = sucrose laurate ester, PEG-8-L = octaoxyethylenelaurate-ester.

[b] as previously assessed by Van den Bergh et al. using extrusion, electron spin resonance and electron microscopy (14). Vesicle elasticity increased with increasing amounts of PEG-8-L. Vesicles were considered to be elastic when it was possible to extrude the formulations through membranes with pore sizes of 30 nm. Rigid vesicles could not be extruded through membranes with pore sizes of 30 nm.

United Kingdom). All measurements were done at 27°C at an angle of 90° between laser and detector. Before measuring, all samples were diluted with a 0.05M citrate buffer solution at pH 5.0 to prevent multiple scattering. All vesicle formulations were measured over a period of 14 days. Vesicles were considered to be stable if their sizes did not change significantly within 14 days.

Visualization with Cryo-Transmission Electron Microscopy (Cryo-TEM)

Pergolide-loaded vesicle and micelle solutions were visualized by the Cryo-TEM method previously described by Frederik *et al.* (18). Briefly, 700 mesh grids were quickly dipped and withdrawn from the sample solutions. Thereafter, excess solution was blotted away with filter paper, leaving thin sample films spanning the grid holes. These films were vitrified by plunging the grid into ethane, which was kept at its melting point by liquid nitrogen. The vitrified sample films were transferred to a Philips CM12 microscope using a Gatan cryotransfer system (Philips, The Netherlands) and visualized at a temperature between −170°C and −173°C.

In Vitro Transport Studies

In vitro transport studies were performed using flow-through Franz diffusion cells. Dermatomed fresh human skin (200μm–300μm) was used, which was obtained after abdominal or mammary cosmetic surgery. All experiments were performed within 24 h of surgical removal of the skin. The donor compartment consisted of 20 μl of the test vesicle or micelle solution. This was applied non-occlusively to a diffusion surface area of 1.1 cm^2. In addition, a saturated buffer solution of pergolide (pH 5.0) was applied to serve as a control. The acceptor compartment consisted of a phosphate buffered saline pH 7.4, which was perfused at a flow-rate of 1.2 ml/h. The acceptor compartment was kept at 37°C and the skin surface was kept at 32°C. Samples were collected every 2 h over a total period of 46 h.

HPLC Assay

Samples obtained from the *in vitro* transport studies were analyzed for pergolide using a reverse-phase HPLC assay. The HPLC system consisted of a Gynkotek P580 HPLC pump equipped with a Gilson Model 231 automatic injector and a FF920 Jasco fluorescence detector. The excitation wavelength was set at 280 nm, the emission wavelength at 346 nm. Samples were injected onto a Prontosil Eurobond C18 (125 × 4.0mm, 5μm) column and eluted at a flow-rate of 1ml/min. The mobile phase consisted of a 0.8% triethanolamine/water mixture (pH 2.85 with phosphoric acid) and acetonitrile at a ratio of 6:9 (v/v). A series of standards was run with each series of samples. The retention time of pergolide was 2.7 min.

Data Analysis of in Vitro Transport Studies

Cumulative amounts vs. time plots were used to calculate the steady-state flux (J_{ss}) and the lag-time (t_{lag}). All statistical analyses were carried out using unpaired two-tailed Students *t* tests. The significance level was set at $P < 0.05$.

RESULTS

Solubilization of Pergolide into Vesicle and Micelle Solutions

Figure 1 shows the maximum amount of pergolide that could be dissolved plotted against the PEG-8-L content of the vesicular system. It can be seen that 0.15% (w/v) of pergolide could be dissolved into the most rigid vesicles (PEG-8-L content of 0 mol%). Increasing the PEG-8-L content—and thereby increasing the vesicle elasticity—from 0 mol% to 30 mol% resulted in a higher drug solubility. The maximum drug solubility of 0.35% was achieved when L-595/PEG-8-L (70/30) elastic vesicles were used. However, a further increase in the PEG-8-L content resulted in a decrease in drug solubility, giving a maximum drug solubility of 0.25% for the most elastic vesicles L-595/PEG-8-L (50/50) and the micelle solution.

Vesicle Size and Stability

Figure 2 shows the sizes of different pergolide-loaded vesicle formulations measured over a period of 14 days. On day 1, the mean diameters of all vesicles were in the range of 100–140 nm. Results were very reproducible as is apparent from the small variations. Over the course of 14 days, only L-595/Peg-8-L (90/10) rigid vesicles increased significantly in size. However, the increase was only 6.8% of their original sizes on day 1. All vesicle formulations were therefore considered to be stable for 14 days. The polydispersity index of all vesicle formulations was between 0.2 and 0.4, indicating that the solutions were moderately homogeneous (0.0 is very homogeneous, 1.0 is very heterogeneous).

The sizes of the micelles were not investigated, as this was beyond the detection limit of our DLS equipment. However, it is worth to mention that the micelle solutions were colloidally unstable for changes in temperature.

Visualization of Pergolide-Loaded Vesicle and Micelle Solutions

The cryo-TEM results, shown in Fig. 3a–3e, confirm the presence of vesicles in all formulations. All vesicle compositions had similar sizes and homogeneity, which is in agreement with the results obtained from DLS measurements. The rigid L-595/PEG-8-L (100/0) and (90/10) compositions contained mainly uni- and bilamellar vesicles (Fig. 3a–3b). Some vesicles had an elongated shape. Increasing the PEG-8-L con-

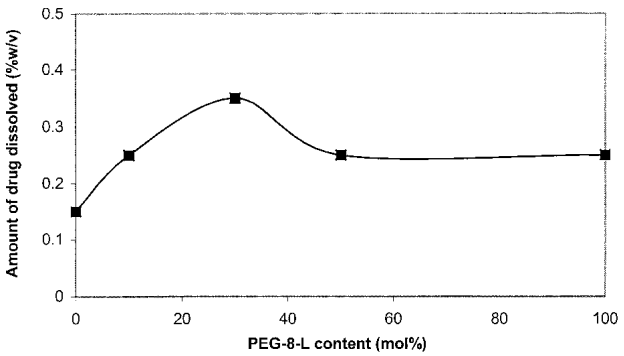

Fig. 1. Effect of vesicle composition on the solubility of pergolide. Data are presented as the mean (n = 2). Error bars were within the size of the symbol and therefore not shown.

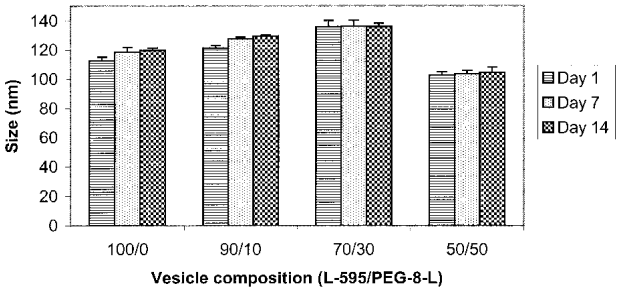

Fig. 2. Stability of L-595/PEG-8-L rigid and elastic vesicles. Vesicles sizes did not increase significantly over a period of 14 days. Data are shown as mean (n = 4) ±SEM.

tent gave several changes in vesicle morphology. The L-595/PEG-8-L (70/30) composition appeared as spherical unilamellar vesicles (Fig. 3c). At this PEG-8-L content of 30 mol%, vesicles were seen as "closed" structures and no micelles could be observed. However, increasing the PEG-8-L content to 50 mol% resulted in "open" vesicles. Vesicle bilayers frequently showed perforations and holes (Fig. 3d). In addition, bilayer fragments were observed. A further increase in the PEG-8-L content will result in vesicle-micelle transition. Figure 3e shows a micrograph of the pergolide-loaded micelle solution, showing the presence of thread-like micelles.

In vitro Transport Studies

Four different pergolide-loaded vesicle compositions were investigated, ranging from very rigid to very elastic vesicles. In addition, PEG-8-L micelles were also studied. A saturated buffer solution of pergolide served as the control. Figure 4 plots the flux of pergolide vs. the time for the different treatments mentioned above. The permeation parameters are summarized in Table II. Large variations between different experiments were observed. This was due to the inherent variations between skin samples from different donors. However, the enhancement effect of each treatment within each experiment was consistent.

As can be seen from Fig. 4, pergolide slowly diffuses across human skin, reaching a steady state not until 25–30 h after application. The steady state was reached earlier with the buffer treatment compared to the vesicle and micelle formulations. Table II shows that all vesicle and micelle compositions had a longer t_{lag} compared to the pergolide buffer solution. This difference was found to be significant for the L-595/PEG-8-L (70/30) and the L-595/PEG-8-L (50/50) elastic vesicles ($P < 0.01$ in both cases). There was no significant difference in t_{lag} between the different vesicle and micelle treatments ($P > 0.05$ in all cases).

As can be deduced from Fig. 4 and Table II, the transport of pergolide was influenced by vesicle treatment and greatly enhanced when applied with elastic vesicles. It was found that the most rigid vesicles L-595/PEG-8-L (100/0) significantly reduced the J_{ss} by half compared to the buffer control ($P < 0.05$). However, increasing the PEG-8-L content—and thereby increasing the vesicle elasticity—resulted in a significant higher J_{ss} for the L-595/PEG-8-L (70/30) elastic vesicles ($P < 0.01$). This was a three-fold increase to the buffer treatment and a six-fold increase to the most rigid vesicle treatment. Further increase in the PEG-8-L content slightly decreased the J_{ss}. However, the L-595/PEG-8-L (50/50) elastic vesicles and the PEG-8-L micelles also showed an increased drug transport by more than a factor 2 compared to the buffer control.

DISCUSSION

In the present study, we investigated the effect of a series of L-595/PEG-8-L vesicles, ranging from very rigid to very elastic. This enabled us to select the best vesicle composition, giving the optimum balance between vesicle stability, elasticity and effectiveness as a skin delivery vehicle.

L-595/PEG-8-L vesicles have previously been studied in the absence of drugs. In this study, the model drug pergolide was dissolved into L-595/PEG-8-L elastic and rigid vesicle formulations.

Characterization and Visualization Studies

L-595/PEG-8-L vesicles in the absence of drugs have previously been characterized and visualized by Van den Bergh *et al.* (13). However, the addition of drug molecules could potentially alter the physico-chemical characteristics as well as the morphology of the vesicular system. Pergolide was dissolved into vesicle and micelle formulations at saturated concentrations to obtain equal and maximum thermodynamic activities. This resulted in an equal driving force for partitioning of the drug from the formulation into the skin. At the pH of 5 used in this study, the drug was associated with the vesicles as well as dissolved in the aqueous phase. Since saturated concentrations of pergolide in the vesicle solutions were far above its saturation concentration in the buffer solution, it was assumed that most of the drug was associated with the lipid bilayers.

Our objectives were to obtain the above mentioned saturated concentrations while maintaining vesicle size, stability, and elasticity. It should be stressed that these factors are closely linked and strongly depend on the ratio between the different vesicle components.

Vesicle stability can be increased by the addition of charged molecules, which causes repulsion forces thereby preventing vesicle fusion. Vesicles in this study contained the negatively charged stabilizer sulfosuccinate and the positively charged model-drug pergolide. Therefore, a certain ratio was required between the sulfosuccinate and the pergolide to obtain a net charge sufficient to maintain vesicle stability. Furthermore, PEG-8-L also plays a role in stabilization. Its large headgroups induces entropic stabilization and prevents vesicle fusion. Due to this additional stabilization, the addition of PEG-8-L reduces the net charge needed to maintain vesicle stability. The second importance of PEG-8-L to is increase vesicle elasticity. However, too high concentrations of PEG-8-L will destabilize the vesicular system and induce micelle formation. Hence, there is an optimum concentration of PEG-8-L that gives the optimal balance between vesicle stability and elasticity, without the formation of micelles.

The rigid vesicles L-595/PEG-8-L (100/0) and (90/10) contained little or no PEG-8-L for stabilization. Therefore, higher amounts of sulfosuccinate were required to give the sufficient negative net charge for stable vesicles. Increasing the PEG-8-L content increases both vesicle elasticity as well as vesicle stability, thereby reducing the net charge required

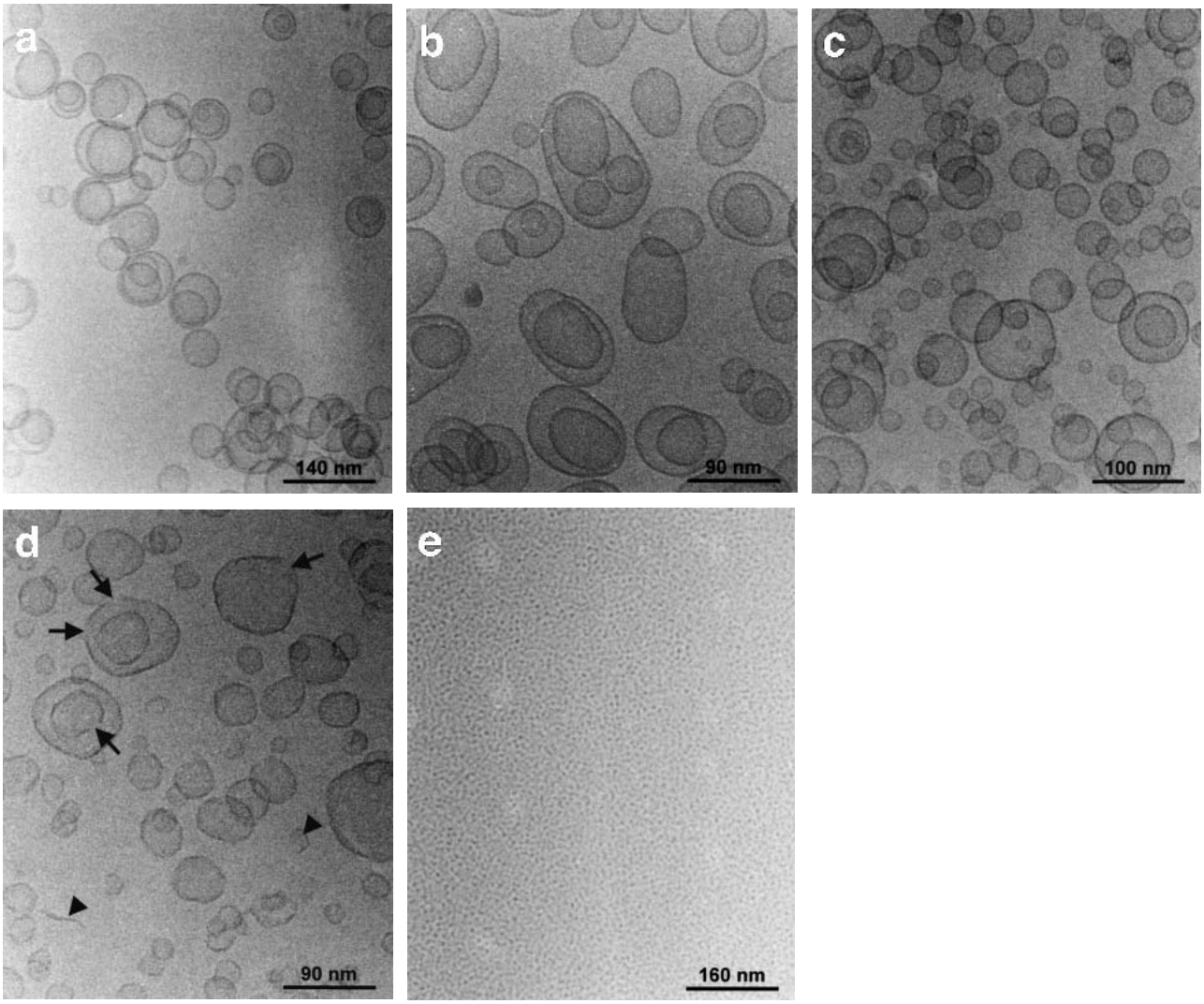

Fig. 3. Micrographs of L-595/PEG-8-L elastic and rigid vesicles. Figure 3a and 3b depict respectively L-595/PEG-8-L (100/0) and (90/10) vesicles, showing uni- and bilamellar vesicles. L-595/PEG-8-L (70/30) vesicles were seen as spherical unilamellar closed structures (Figure 3c). Fig. 3d depicts L-595/PEG-8-L (50/50) vesicles. Open vesicles with perforated bilayers can be observed (arrows). In addition, bilayer fragments were frequently seen (arrowheads). Further increase in the PEG-8-L content resulted in a transition from vesicles to micelles (Fig. 3e).

to maintain stable vesicles. This would reduce the amount of sulfosuccinate needed and increase the amount of pergolide that could be added. The highest drug solubility was achieved using L-595/PEG-8-L (70/30) elastic vesicles. A further increase in the PEG-8-L content resulted in a decrease in drug solubility. A further increase in the PEG-8-L content also destabilize the vesicular system as was confirmed by the Cryo-TEM micrographs. Cryo-TEM results clearly demonstrated the breakdown of vesicles into bilayer fragments at a PEG-8-L content of 50 mol%, indicating the first step towards vesicle-micelle transition.

Vesicle sizes, stability and morphology in this study were similar to those found for L-595/PEG-8-L vesicles in the absence of drugs (13). We were able to add saturated concentrations of pergolide without affecting the main physicochemical characteristics of the vesicular system. The optimal vesicle composition of L-595/PEG-8-L (70/30) gave the best balance between vesicle stability and elasticity, as well as the highest drug solubility.

In Vitro Transport Studies

In vitro flow-through Franz-cell measurements were used to investigate the transport enhancement potential of elastic and rigid vesicles. This method is commonly used in skin research and normally gives a good correlation with the *in vivo* data. It is believed that a transepidermal osmotic gradient is the driving force for elastic vesicle transport (19). This osmotic gradient is present *in vitro* (unpublished results) and was maintained during our experiments by non-occlusive application. However, it should be mentioned that other factors could contribute to the mechanisms of action of elastic vesicles. It is yet unclear what these factors are and to what extent they are present in the *in vitro* and *in vivo* situation. Therefore, future *in vivo* experiments in humans are required to further assess the transport enhancement potential of elastic and rigid vesicles.

The results of these studies showed that pergolide slowly diffuses across human skin, reaching steady-state fluxes of

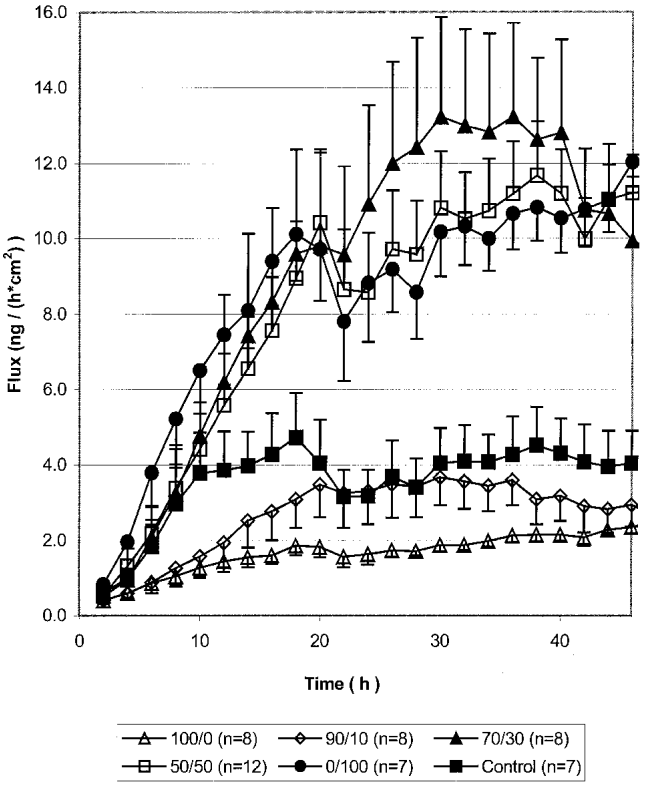

Fig. 4. Flux of pergolide from different vesicle formulations and the saturated buffer control. Data are presented as mean + SEM.

2–14 ng/(h*cm^2). From these results, it is expected that clinically relevant pergolide plasma concentrations would not be reached following transdermal vesicular delivery. However, it should be noted that the partitioning of pergolide in the aqueous acceptor phase of pH 7.4 is very poor. In the *in vi*vo situation, pergolide is for 90% bound to human plasma proteins, which enables the transport of this drug in blood at the same pH. It can therefore be assumed that the fluxes obtained in this study are lower than would be obtained in the *in vivo* situation.

The results obtained in this study clearly showed that elastic vesicles enhanced the transport of pergolide, while rigid vesicles impeded the transport of pergolide. These findings are in agreement with the results found by Van den Bergh *et al.*, who determined the penetration of ^3H_2O after pretreating mouse skin with "empty" elastic and rigid vesicles (15). Several other papers have also shown that elastic vesicles are far superior to rigid vesicles (8–12,20).

Although elastic vesicles gave higher drug transport, the rate of drug transport was diminished, as was evident from the longer lag-times obtained. This suggests that elastic vesicles in the skin acted as a slow release depot system. The release of vesicle-associated pergolide was slow due to its high solubility in lipids. Despite a longer time to reach the desired effect, depot formation is of advantage to a delivery system that requires a constant controlled release.

To date, the mechanism of action of elastic vesicles is still a subject of discussion. One can debate that the enhancement of drug transport is simply caused by a penetration enhancing effect of the PEG-8-L. If this were to be true, we would expect the maximum transport to be given by the PEG-8-L micelles. However, the maximum transport was given by L-595/PEG-8-L (70/30) elastic vesicles. El Maghraby *et al.* have found that too low or too high concentrations of the membrane-solubilizing components were not beneficial in vesicular delivery of oestradiol (10). They, therefore, suggested that the possible penetration enhancing effect of these components is not mainly responsible for the improved oestradiol skin delivery from elastic vesicles. Van den Bergh *et al.* investigated the effect of vesicle and micelle treatment on the penetration of a fluorescent label into human skin *in vitro* (6). After treatment with elastic vesicles, flourescent material was localized in a fine meshwork of channels-like structures. In contrast, after treatment with PEG-8-L micelles, there was a homogeneous intercellular penetration of the fluorescent label. Honeywell-Nguyen *et al.* have investigated the *in vivo* interactions between vesicles and human skin by visualizing vesicle-treated skin using the tape-stripping technique combined with electron microscopy. In skin treated with elastic vesicles, vesicular structures were found up to the ninth tape-strip, where they accumulated in channel-like regions (21). In contrast, skin treated with micelles showed rough, irregular fracture planes (unpublished results). These results suggest that elastic vesicles and micelles both enhance skin permeability, however, most likely in a different way. Whether elastic vesicles accumulate in channel-like regions and thereby create penetration pathways, or whether other mechanisms are involved, is still not fully understood. The exact mechanism of action is currently a subject of investigation and will be presented in the near future.

CONCLUSIONS

The results of this study demonstrate the importance of vesicle composition on the physico-chemical properties, morphology, drug solubility, and consequently on the effectiveness of the vesicular system as a vehicle for transdermal drug delivery. Elastic vesicles were significantly better compared to rigid vesicles. The best vesicle composition was found to be L-595/PEG-8-L (70/30) elastic vesicles. These vesicles gave the best balance between vesicle stability, elasticity, drug solubility, as well as the highest enhancement of drug transport across human skin.

The results of this study support the hypothesis that elastic vesicles are promising tools for transdermal drug delivery. However, their mechanism of action needs to be unraveled in order to develop and optimize suitable transdermal drug applications.

Table II. Permeation Parameters of Pergolide from Different Vesicle and Micelle Treatments as Compared to a Saturated Buffer Control[a]

Vesicle/micelle composition (L-595/PEG-8-L)	Cumulative amount after 46 h(ng/cm^2)	Steady-state flux (ng/(h*cm^2))	Lag-time (h)
Buffer control (n = 7)	163.5 ± 35.9	4.2 ± 0.9	7.6 ± 1.9
100/0 (n = 8)	76.0 ± 6.9	2.2 ± 0.2	12.0 ± 1.5
90/10 (n = 8)	122.5 ± 26.1	3.7 ± 0.7	12.4 ± 1.9
70/30 (n = 8)	413.7 ± 85.5	13.6 ± 2.3	15.9 ± 1.8
50/50 (n = 12)	374.0 ± 51.7	11.5 ± 1.3	14.8 ± 1.4
0/100 (n = 7)	386.0 ± 38.6	10.8 ± 0.6	10.7 ± 2.3

[a] Data are presented as mean ± SEM.

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