Skin Penetration and Mechanisms of Action in the Delivery of the D_2-Agonist Rotigotine from Surfactant-Based Elastic Vesicle Formulations

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Purpose. This study was performed to investigate the effect of elastic and rigid vesicles on the penetration of the D_2 dopamine agonist rotigotine across human skin and to further elucidate the mechanisms of action of the elastic vesicles.

Methods. A series of rotigotine-loaded vesicles were prepared, ranging from very elastic to very rigid. The drug penetration from these vesicles across human skin was studied *in vitro* using flow-through diffusion cells. Micelle and buffer solutions were investigated as controls. For the most elastic vesicle composition, two additional variables were investigated. Coapplication of drug and vesicles was compared to pretreatment, and the effect of the drug entrapment efficiency was investigated.

Results. The very elastic vesicle formulation L-595/PEG-8-L (50/50) gave steady-state fluxes of 214.4 ± 27.8 ng/(h · cm^2). This formulation was the most effective formulation and significantly better than the rigid vesicle formulations as well as the micelle and buffer controls. However, coapplication and a high drug entrapment efficiency were essential factors for an optimal drug delivery from elastic vesicle formulations.

Conclusions. Elastic vesicles are promising vehicles for transdermal drug delivery. It is essential that drug molecules are applied together with and entrapped within the vesicles themselves, suggesting that elastic vesicles act as drug carrier systems and not solely as penetration enhancers.

KEY WORDS: transdermal delivery; elastic vesicles; rotigotine; drug carrier systems.

INTRODUCTION

The use of vesicles to enhance dermal and transdermal drug transport has gained much interest in the last decade (1,2). Recently, a few novel types of vesicles have been designed that have been shown to be more effective than conventional liposomes (3–5).

In the early 1990s, the use of elastic vesicles was introduced. These are a novel type of liquid-state vesicles, characterized by elastic, deformable lipid membranes. The vesicle elasticity was obtained by combining stabilizing and destabilizing molecules within one lipid membrane. The first generation of elastic vesicles, also referred to as Transfersomes®, was designed by Cevc *et al.* (6,7). These vesicles were composed of phosphatidylcholine in combination with the edge activator sodium cholate. Subsequent studies have demonstrated that Transfersomes® were more effective than standard liposomes in the enhancement of lidocaine and insulin absorption across mouse and human skin (8,9). Using the same vesicle composition, El Maghraby *et al.* have later shown that elastic vesicles were also superior to traditional liposomes in the transdermal delivery of estradiol and 5-fluorouracil (10–12).

In 1998, Van den Bergh *et al.* introduced a new series of elastic and rigid vesicles consisting solely of surfactants. These surfactant-based elastic and rigid vesicles consisted of the bilayer-forming surfactant L-595 (sucrose laurate ester) and the micelle-forming surfactant PEG-8-L (octaoxyethylene laurate ester). We have recently demonstrated that L-595/PEG-8-L (50/50) elastic vesicles were more effective than L-595/PEG-8-L (100/0) rigid vesicles in the *in vitro* enhancement of pergolide and lidocaine transport across human skin (13,14).

Despite very encouraging results from transport studies, to date the mechanism of action of elastic vesicles is not yet fully understood. Two mechanisms can be proposed. First, the elastic vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum (SC) and subsequently modify the intercellular lipid lamellae. This will facilitate the penetration of free drug molecules into and across the SC (mechanism 1). Second, the elastic vesicles can act as drug carrier systems, whereby intact vesicles can enter the SC carrying vesicle-bound drug molecules into the skin (mechanism 2). The latter mechanism of action was put forward in 1992 by Cevc *et al.*, who proposed that Transfersomes® are drug carrier systems that can cross the skin intact (7). It is believed that the successful passage of such carriers is based on two important factors: the highly stress-dependent elasticity of the vesicle bilayers and the existence of an osmotic gradient across the skin. Because of their stress-dependent deformability, Transfersomes® could—under influence of the transepidermal water gradient—squeeze themselves between the cells in the SC and carry large amounts of drugs across the intact skin (6). Recently, Honeywell-Nguyen *et al.* have described a drug carrier mechanism of action—different from the one suggested by Cevc *et al.*—for L-595/PEG-8-L elastic vesicles, based on the results of transport studies using pergolide (15) and based on visualization studies (16,17). On the basis of the results of these studies, we propose that elastic vesicles rapidly enter the SC but do not penetrate intact into the viable epidermis.

The aim of the present study was twofold. The first aim was to investigate the effect of L-595/PEG-8-L elastic and rigid vesicles on the *in vitro* transport of rotigotine across human skin. Rotigotine [(−)2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin HCl, also known as N-0923] is a D_2 dopamine agonist used in the treatment of Parkinson's disease (18). A transdermal drug delivery system is of advantage in the management of Parkinson's disease because this could provide a constant drug input. This would significantly reduce the side effects of anti-Parkinson's treatment, which are thought to result from erratic drug plasma levels. Furthermore, rotigotine undergoes extensive gastrointestinal metabolism, making oral administration unsuitable. The second aim of this study was to further elucidate the mode of action of the elastic vesicles, focusing on the two mechanisms of

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action mentioned above. In order to assess whether a drug carrier mechanism of action is involved or whether elastic vesicles simply act as penetration enhancers, two important questions should be answered: (a) Is pretreatment of the skin with empty vesicles sufficient (indicative of mechanism 1), or is it essential to incorporate drugs into the vesicle solution (indicative of mechanism 2)? (b) What is the effect of the entrapment efficiency on the drug transport? Does a higher entrapment efficiency result in a higher drug transport?

In order to answer the first question, the difference between elastic vesicle pretreatment and elastic vesicle coapplication was investigated. In order to answer the second question, a comparison was made between the drug transport from two elastic vesicle formulations having equal thermodynamic activities but different entrapment efficiencies.

MATERIALS AND METHODS

Materials

L-595 consisted of 100% sucrose laurate ester (30% mono-, 40% di-, and 30% triester) and was kindly supplied by Mitsubishi Kasei (Tokyo, Japan). The octaoxyethylene laurate ester (PEG-8-L) was a gift from Lipo Chemicals (Paterson, NJ, USA), and the sodium sulfosuccinate was a gift from Cytec (Rotterdam, The Netherlands). Rotigotine was very kindly provided by Schwarz Pharma (Monheim, Germany). In all experiments, the pH 5.0 buffer was a 0.05 M citrate buffer, and the pH 9.0 buffer was a 0.05 M borax buffer.

Vesicle Composition

A series of surfactant-based vesicles was prepared, ranging from very rigid to very elastic. These vesicles consisted of the bilayer-forming surfactant L-595 and the micelle-forming surfactant PEG-8-L. The incorporation of PEG-8-L would increase the elasticity of the vesicles. The compositions and molar ratios of the formulations used in this study are shown in Table I. In a previous study vesicle elasticity and morphology were assessed using extrusion, electron spin resonance, and electron microscopy (19). Vesicles were considered to be very elastic when it was possible to extrude the formulations through membranes with pore sizes of 30 nm. Rigid vesicles could not even be extruded through membranes with pore sizes of 50 nm. A small amount of sulfosuccinate was added to the elastic and rigid vesicles in order to increase vesicle stability. However, for clarity, all vesicle compositions will be indicated below 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. In addition to elastic and rigid vesicles, PEG-8-L micelles were also investigated as a comparison.

A series of rotigotine-loaded vesicle and micelle formulations were prepared at pH 9.0. In addition, the L-595/PEG-8-L (50/50) elastic vesicle composition was also prepared at pH 5.0. These two pH values were chosen based on the pK_a of rotigotine, which is 7.9. At pH 9.0 rotigotine is very lipophilic and hence expected to be bound to the vesicle bilayers to a high degree (high entrapment efficiency). In contrast, at pH 5.0 rotigotine is hydrophilic and hence expected to be mainly present in the water phase of the drug-vesicle formulation (low entrapment efficiency).

Incorporation of Rotigotine into Vesicle and Micelle Solutions

Rotigotine was incorporated into the vesicle or micelle solutions at maximum concentrations at which stable formulations could still be obtained. To determine these concentrations, increasing amounts of rotigotine were added during

Table I. Composition of Vesicle and Micelle Formulations and Their Characterization for Size, Stability, and Entrapment Efficiency

Composition (L-595/PEG-8-L/sulfosuccinate)*	Elasticity/rigidity†	Total drug concentration in vesicle formulation (mg/ml)‡	pH	Vesicle size on day 1 (nm)§	Vesicle size on day 14 (nm)§	Entrapment efficiency (%)‖
To investigate the effect of vesicle composition						
100/0/5	Very rigid	6.7	9.0	120 ± 7	128 ± 6	ND**
90/10/5	Moderately rigid	6.7	9.0	111 ± 7	130 ± 9	ND**
70/30/5	Moderately elastic	8	9.0	112 ± 7	1117 ± 7	ND**
50/50/5	Very elastic	12	9.0	117 ± 6	133 ± 8	99.8 ± 0.02
0/100/0	Micelles	5.3	9.0	NM¶	NM¶	ND**
Buffer control	Not applicable	0.037	9.0	Not applicable	Not applicable	Not applicable
To investigate the effect of entrapment efficiency						
50/50/5	Very elastic	12	9.0	117 ± 6	133 ± 8	99.8 ± 0.02
50/50/5	Very elastic	12	5.0	112 ± 1	115 ± 6	21.1 ± 9.6
Buffer control	Not applicable	0.036	9.0	Not applicable	Not applicable	Not applicable
Buffer control	Not applicable	4.7	5.0	Not applicable	Not applicable	Not applicable

* L-595, sucrose laurate ester; PEG-8-L, octaoxyethylenelaurate ester.

† As previously assessed by Van den Bergh *et al.* using extrusion, electron spin resonance, and electron microscopy (19).

‡ The total drug concentration in the vesicle formulation is 66.7% of the saturation concentration for all formulations. Hence, all formulations had an equal thermodynamic activity.

§ Data are presented as mean ± SEM (n = 3).

‖ Data are presented as mean ± SEM (n = 3 or n = 5 for formulations at pH 5.0 and pH 9.0, respectively).

¶ NM, not measurable.

** ND, not determined.

preparation of the vesicle or micelle solutions. It was assumed that the presence of rotigotine crystals, which were looked for over a period of 14 days using light microscopy, would indicate that the solution was saturated. Vesicle stability studies were performed simultaneously as described below.

The drug concentrations used are shown in Table I. These concentrations are not saturated concentrations. Vesicle and micelle solutions could not be prepared in the presence of saturated concentrations of rotigotine, as these produced unstable formulations. The drug concentrations in all vesicle and micelle formulations were 66.7% of the saturation concentrations. At these concentrations, all vesicle and micelle formulations were found to be stable for at least 14 days. Furthermore, all formulations had equal thermodynamic activities. Hence, they can be compared to each other in transport studies because the driving forces for rotigotine to partition from the formulation into the skin were equal in all cases.

Preparation of Rotigotine-Loaded Vesicle and Micelle Formulations

Vesicles were prepared by a modification of the sonication method described by Baillie *et al.* (20). Briefly, the surfactants and rotigotine were dissolved in ethanol, while sulfosuccinate was dissolved in an ethanol/isopropanol mixture. The solutions were mixed together in appropriate ratios. The organic solvents were then evaporated overnight in a vacuum centrifuge, and the remaining lipid film was rehydrated with a buffer solution at pH 9.0. In addition, the L-595/PEG-8-L (50/50) elastic vesicle formulation was also prepared at pH 5.0. Subsequently, vesicle solutions were sonicated using a Branson Sonifier 250 (Branson Ultrasonics Corp., Danbury, UK) with a 7-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 surfactants.

Size and Stability Studies

The z-average diameters of all vesicle formulations containing rotigotine were measured by dynamic light scattering using a Malvern Zetasizer 3000 HS_A (Malvern Ltd., Malvern, UK). All measurements were done at 27°C at an angle of 90° between laser and detector. Before measuring, samples were appropriately diluted with a buffer solution at pH 9.0 or pH 5.0 to prevent multiple scattering. The vesicle formulations were measured over a period of 14 days, and they were considered to be stable if their sizes did not change significantly within this period of time.

Determination of the Entrapment Efficiency

The entrapment efficiency was determined for the L-595/PEG-8-L (50/50) elastic vesicle composition at pH 9.0 and pH 5.0. During the incorporation studies, both solutions at pH 9.0 and 5.0 were, coincidentally, found to have the same saturation concentration of 18 mg/ml. Because 66.7% of the saturation concentration was used in all studies, these two formulations had an equal total drug concentration of 12 mg/ml (see Table I). However, based on the solubility of rotigotine at pH 9.0 and pH 5.0, very different entrapment values can be expected.

The entrapment efficiency was determined using a microdialysis method. Dialysis fibers (Gambro GFS +12, Gambro Dialysaten, Hechingen, Germany) with an inner diameter of 200 μm and membrane pores of 2000 d were used. The dialysis fibers were placed into the elastic vesicle solutions over a length of 3 cm. The fibers were perfused with a buffer solution at pH 9.0 or pH 5.0 using a flow rate of 250 μl/h. Because of the molecular cutoff of 2000 d, free drug will be able to pass the dialysis fiber membrane, but vesicles and vesicle-bound drug are not able to do so. After 30 min of equilibration, the perfusate was collected over a period of 1 h and analyzed for the concentration of free drug using HPLC.

At a constant flow rate in the steady-state situation, there is a constant relationship between the concentration of free-drug sampled in the perfusate and the concentration of free-drug in the vesicle solution:

$$C_{\text{free-drug sampled in perfusate}}/C_{\text{free-drug in vesicle solution}} = \text{Constant R at a constant flow rate} \quad (1)$$

Hence, each experiment was performed with a series of "standards" in which the vesicle solutions were replaced by solutions with known concentrations of rotigotine. A linear relationship was obtained between these known concentrations and the sampled concentrations of rotigotine in the perfusate. Constant R was then calculated from the slope of the straight line when the sampled concentrations in the perfusate were plotted as a function of the known concentrations of rotigotine. Subsequently, the free drug and vesicle-bound drug concentrations in the vesicle solutions were calculated by:

$$C_{\text{free-drug in vesicle solution}} = C_{\text{free-drug sampled in perfusate}}/\text{Constant R} \quad (2)$$

$$C_{\text{vesicle-bound drug in vesicle solution}} = C_{\text{total drug added to vesicle solution}} - C_{\text{free-drug in vesicle solution}} \quad (3)$$

The entrapment efficiency was determined as the percentage of drug that was bound to the vesicles.

In Vitro Transport Studies

In vitro transport studies were performed using flow-through Permegear diffusion cells. Fresh human skin was obtained from abdominal or mammary cosmetic operations. All experiments were performed within 24 h of surgical removal of the skin. After removal of the subcutaneous fat tissue, the skin was dermatomed to a thickness of 200–300 μm. The donor compartment consisted of 20 μl of the test vesicle, micelle, or buffer formulation, which was applied nonocclusively to a skin surface area of 1.1 cm^2. If not specified the formulations were at pH 9.0. A nonocclusive application was used because this is necessary in order to create a transepidermal osmotic gradient, which has been suggested to be the driving force for the transport of elastic vesicles into the skin (7). The acceptor phase consisted of phosphate-buffered saline, pH 7.4, perfused at a constant flow rate of 1.2 ml/h. The acceptor compartment was stirred continuously and was kept at a temperature of 37°C. The latter resulted in a skin surface temperature of 32°C. Samples were collected every hour for a total period of 24 h.

Three different sets of experiments were performed.

The Effect of Vesicle Composition

A series of L-595/PEG-8-L vesicles were investigated, ranging from very rigid to very elastic (see Table I). In addition, PEG-8-L micelles and a 66.7% saturated buffer solution were investigated as comparison.

The Effect of Coapplication and Pretreatment

These experiments were performed using the L-595/PEG-8-L (50/50) elastic vesicles. Pretreatment of the skin involved nonocclusive application of 20 μl of "empty" elastic vesicles for 1 h. Thereafter, the donor compartment was rinsed, and 20 μl of a 66.7% saturated buffer solution of rotigotine at pH 9.0 was applied. Pretreatment was compared to coapplication, which involved the application of 20 μl of a rotigotine-loaded elastic vesicle solution. Rotigotine transport from a buffer solution without previous vesicle pretreatment was investigated as the control. A pretreatment period of 1 h was chosen because previous studies have reported that the *in vivo* partitioning of elastic vesicles into human SC occurred within 1 h (16), and an increase in the treatment period from 1 h to 16 h did not affect the penetration of a model fluorescent label incorporated into elastic vesicles (21).

The Effect of the pH of the Drug–Vesicular System

Rotigotine-loaded L-595/PEG-8-L (50/50) elastic vesicle solutions at pH 9.0 and pH 5.0 were applied to human skin and compared to each other. These formulations had equal thermodynamic activities but very different drug entrapment efficiencies (see below). In addition, 66.7% saturated buffer solutions of rotigotine at pH 9.0 and pH 5.0 were also investigated as controls.

HPLC Analysis

Samples were analyzed for rotigotine 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 FF821 Jasco fluorescence detector. The excitation wavelength was set at 270 nm, the emission wavelength at 305 nm. Samples were injected onto a Supersper® 60 RP-select B (75 mm – 4) column and eluted at a flow rate of 1 ml/min. The mobile phase consisted of acetonitrile/0.1 M acetate buffer (pH 3.6) at a ratio of 4:6 (v/v). A series of standards was run with each series of samples.

Data Analysis and Statistics

Cumulative amounts vs. time plots were used to calculate the steady-state flux (J_{ss}) and the lag time (T_{lag}). The statistical analyses were performed using unpaired two-tailed Student's *t* tests. Significance was set to $p < 0.05$.

RESULTS

Size and Stability Studies

Table I shows the average sizes of the different rotigotine-loaded vesicle formulations measured over a period of 14 days. On day 1, the mean diameters of all vesicles were in the range of 100–120 nm, with no significant differences between different formulations. Over the course of 14 days, none of the vesicles showed a large increase in size. It can therefore be concluded that rotigotine-loaded elastic and rigid vesicle formulations were stable for 14 days at a drug saturation rate of 66.7%. The sizes of the micelles were not investigated, as this was beyond the detection limit of our DLS equipment.

Entrapment Efficiency

The entrapment efficiency was determined for L-595/PEG-8-L (50/50) elastic vesicle formulations at pH 9.0 and pH 5.0. The results have shown that their entrapment values are significantly different. At pH 9.0, the majority of the rotigotine molecules are bound to the vesicle bilayers, hence an entrapment efficiency of 99.8%. At pH 5.0, however, the entrapment efficiency is very low, suggesting that most of the rotigotine molecules were dissolved in the water phase of the vesicle formulation.

***In Vitro* Transport Studies**

The Effect of Vesicle Composition

In Fig. 1 the transdermal flux of rotigotine is plotted as a function of time for the different vesicle, micelle, and buffer

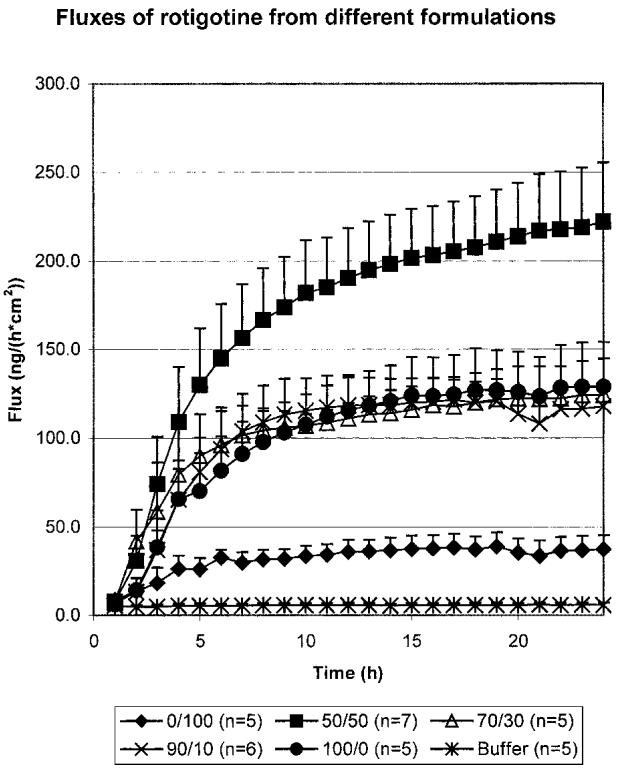

Fig. 1. Fluxes of rotigotine from different elastic and rigid vesicle, micelle, and buffer formulations. The compositions of the vesicle and micelle formulations are given in the legend as the molar ratio between the two main components (L-595/PEG-8-L). Very rigid vesicles consisted only of L-595 (100/0), whereas micelles consisted only of PEG-8-L (0/100). It is evident that all vesicle formulations were more effective than the micelle formulation and the buffer control. However, the best formulation for the enhancement of rotigotine transport across human skin is the L-595/PEG-8-L (50/50), the most elastic vesicle formulation, which results in a factor of 30 transport enhancement as compared to the buffer control. Data are presented as mean ± SEM.

Table II. Permeation Parameters of Rotigotine from Different Vesicle, Micelle, and Buffer Formulations*

Composition (L-595/PEG-8-L/sulfosuccinate)†	Elasticity/rigidity‡	pH	Steady-state flux [ng/(h · cm^2)]	Lag time (h)	Cumulative amount after 24 h (ng/cm^2)
The effect of vesicle composition					
100/0/5 (n = 5)	Very rigid	9.0	129 ± 24	5.2 ± 1.5	2402 ± 356
90/10/5 (n = 6)	Moderately rigid	9.0	120 ± 14	4.1 ± 0.8	2387 ± 321
70/30/5 (n = 5)	Moderately elastic	9.0	124 ± 18	4.1 ± 1.7	2450 ± 156
50/50/5 (n = 7)	Very elastic	9.0	214 ± 28	5.5 ± 1.0	4064 ± 630
0/100/0 (n = 5)	Micelles	9.0	38 ± 8	3.9 ± 1.3	762 ± 113
Buffer solution (n = 5)	Not applicable	5.0	5 ± 1[d]	ND‖	133 ± 27
The effect of coapplicable and pretreatment					
50/50/5 (n = 5) Co-application	Very elastic	9.0	193 ± 54	6.5 ± 0.6	3483 ± 1067
50/50/5 (n = 7) Pre-treatment	Very elastic	9.0	5.2 ± 0.7§	ND‖	126 ± 18
Buffer solution (n = 5)	Not applicable	9.0	5.6 ± 1.1§	ND‖	133 ± 27
The effect of the entrapment efficiency					
50/50/5 (n = 6) High entrapment	Very elastic	9.0	180 ± 46	6.4 ± 0.5	3251 ± 902
50/50/5 (n = 7) Low entrapment	Very elastic	5.0	65 ± 11	7.0 ± 1.4	1072 ± 160
Buffer solution (n = 8)	Not applicable	9.0	2.2 ± 1.4§	ND‖	42 ± 29
Buffer solution (n = 8)	Not applicable	5.0	64 ± 13	4.4 ± 0.7	1133 ± 241

* Data are presented as mean ± SEM.
† L-595, sucrose laurate ester; PEG-8-L, octaoxyethylenelaurate ester.
‡ As previously assessed by Van den Bergh *et al.* using extrusion, electron spin resonance, and electron microscopy (19).
§ Values are below the detection limit of the analysis system and therefore cannot be considered to be accurate.
‖ ND, not determined. Lag times could not be determined because of the very low fluxes obtained (below detection limit).

formulations. The permeation parameters are summarized in Table II. From the results it is evident that the L-595/PEG-8-L (50/50) very elastic vesicle composition is the most effective formulation for the transport of rotigotine across human skin. This formulation was significantly better than the more rigid L-595/PEG-8-L (100/0), (90/10), and (70/30) formulations ($p < 0.05$), enhancing the drug transport by a factor of 1.7. No significant differences were found among the L-595/PEG-8-L (100/0), (90/10), and (70/30) compositions ($p > 0.05$). However, from Fig. 1 and Table II it is very clear that all elastic and rigid vesicle solutions were significantly better than the micelle and buffer solutions ($p < 0.01$). The L-595/PEG-8-L (50/50) very elastic vesicle composition enhanced the drug transport by factors of 5.3 and 30.6 as compared to the micelle and the buffer solutions, respectively. Using this most effective vesicle composition, a J_{ss} of 214.4 ± 27.8 ng/(h·cm^2) was achieved. No significant differences were found in the T_{lag} values for any of the compositions.

Coapplication vs. Pretreatment

Fig. 2 shows the effect of elastic vesicle coapplication and pretreatment on the transdermal flux of rotigotine. From the results it is beyond doubt that coapplication is essential in order to achieve drug transport enhancement. Coapplication significantly enhanced the drug transport by many fold, whereas pretreatment clearly had no effect on the drug transport as compared to the buffer control ($p > 0.05$). Both vesicle pretreatment and the buffer control resulted in fluxes of approximately 5 ng/(hr·cm^2), which was very low and barely detectable with the HPLC analysis system.

The Effect of the Entrapment Efficiency

Fig. 3 and Table II depict the drug transport from L-595/PEG-8-L (50/50) elastic vesicle formulations at pH 9.0 and 5.0 together with the drug transport from buffer solutions at the same pH values. From the results it can be deduced that a high entrapment efficiency is important in order to achieve the optimal drug enhancement effect. The vesicle solution prepared at pH 9.0 with a high drug entrapment efficiency was clearly the best formulation, giving J_{ss} values that were a

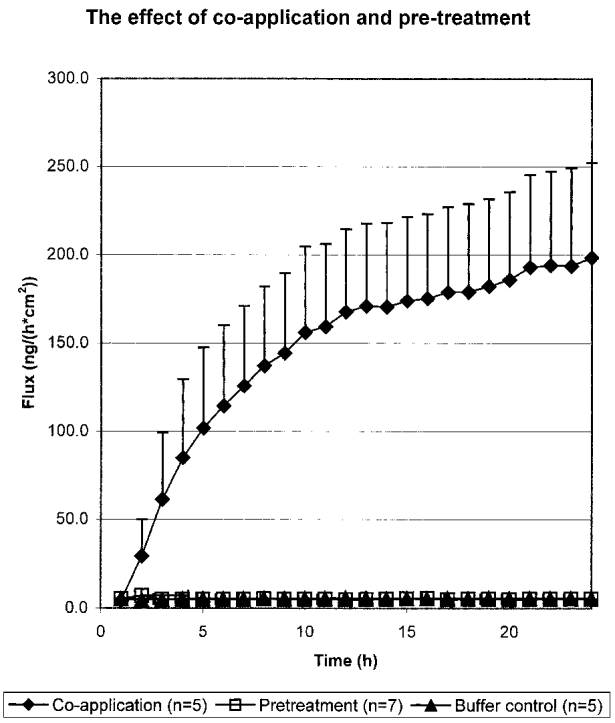

Fig. 2. The effect of L-595/PEG-8-L (50/50) elastic vesicle coapplication and pretreatment on the transport of rotigotine across human skin. Coapplication clearly enhanced the drug transport, whereas pretreatment produced no difference as compared to the buffer control. Data are presented as mean ± SEM.

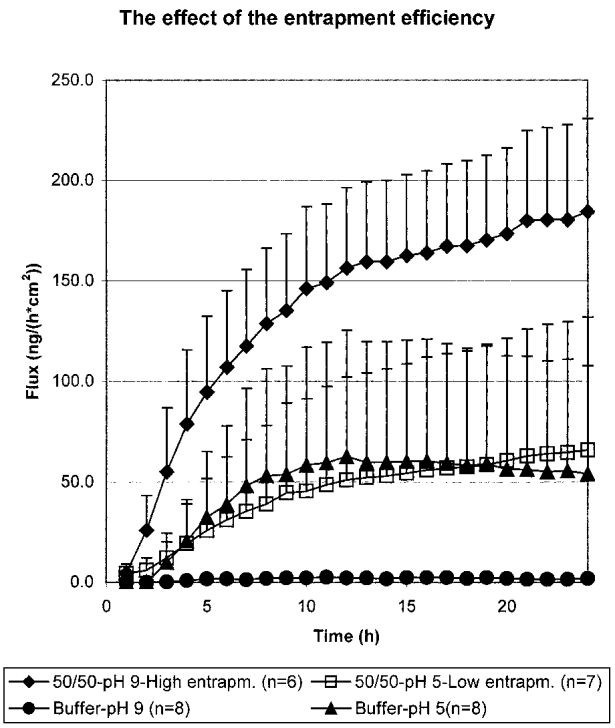

Fig. 3. The effect of the drug entrapment efficiency on the transport of rotigotine from L-595/PEG-8-L (50/50) elastic vesicle formulations. The vesicle formulation at pH 9.0 with a high entrapment efficiency resulted in the highest drug transport, enhancing the transport manyfold as compared to its corresponding buffer control at pH 9.0. The formulation at pH 5.0 with a low entrapment efficiency resulted in a lower drug transport and showed no difference from its corresponding buffer control at pH 5.0. Data are presented as mean ± SEM.

factor 2.7 higher than those resulting from the vesicle solution at pH 5.0 with a low entrapment efficiency. More importantly, vesicles with a high entrapment efficiency at pH 9.0 gave rise to an enhancement effect of factor 80 as compared to the corresponding buffer solution. In contrast, the vesicle solution at pH 5.0 with a low entrapment value did not significantly enhance the drug transport as compared to its corresponding buffer control ($p > 0.05$). No significant differences were found in the T_{lag} values for any of the treatments. Note the difference between the transport from L-595/PEG-8-L (50/50) elastic vesicle and buffer solutions at pH 9.0 in Figs. 1, 2, and 3. This difference was caused by the fact that these data were obtained from three different sets of experiments using skin from three different sets of donors.

DISCUSSION

From our results it is evident that vesicle elasticity has a significant effect on the enhancement of drug transport across human skin. The L-595/PEG-8-L (50/50) elastic vesicle composition was clearly more effective than the more rigid vesicle compositions, the micelle solution, as well as the buffer control. These results are in agreement with previous papers, reporting that the L-595/PEG-8-L elastic vesicles enhanced the *in vitro* transport of pergolide and lidocaine across human skin (13,14). Previous studies have also shown that elastic vesicles prepared with a different composition could significantly enhance transport for a wide variety of other drug compounds (8–12,22). Hence, we can conclude that vesicle elasticity strongly affects the effectiveness of a vesicular system. It should be mentioned that the transdermal fluxes achieved for the most effective elastic vesicle formulation in this study [J_{ss} of 214.4 ± 27.8 ng/(h·cm^2)] do not suggest that plasma concentrations can be achieved at which clinical responses can be expected (23). However, in the *in vivo* situation the solubility and transport of rotigotine in plasma can be strongly enhanced by plasma proteins, which were not present in our acceptor phase. It can therefore be assumed that the fluxes obtained in this study are lower than what would be obtained in the *in vivo* situation.

One of the most interesting issues in vesicular research is to elucidate the mechanisms of action of the elastic vesicles. In the present study, we have focused on two possible mechanisms: (a) elastic vesicles act as penetration enhancers, or (b) elastic vesicles act as drug carrier systems. Based on the results of previous research, these two mechanisms are considered to be the two most likely options. Cevc *et al.* were the first to introduce the concept of elastic vesicles as drug carrier systems. The investigators used Transfersomes®, which were prepared from phosphatidylcholine and sodium cholate. It was proposed that these ultraflexible Transfersomes® were drug carriers that penetrated the skin intact under influence of a transepidermal osmotic gradient (7). Concerning the surfactant-based L-595/PEG-8-L elastic vesicles, previous studies have shown that the (50/50) elastic vesicles were superior to (100/0) rigid vesicles, but no differences were found between the elastic vesicles and PEG-8-L micelles (13,14). One could therefore debate that the enhancement effect of the L-595/PEG-8-L elastic vesicles was entirely a result of the presence of PEG-8-L, suggesting a penetration-enhancing mechanism of this micelle-forming vesicle component. In a recent study using freeze-fracture electron microscopy, however, L-595/PEG-8-L (50/50) elastic vesicles showed a very different interaction with human skin as compared to PEG-8-L micelles, suggesting that these two formulations have different mechanisms of actions within the SC (16). Furthermore, the electron micrographs have shown the presence of intact elastic vesicles in the deeper layers of the SC with no abnormalities in the intercellular lipid lamellae. Hence, these results were already indicative of the fact that elastic vesicles act as a drug carrier system. However, no evidence was found that a substantial number of vesicles entered the viable epidermis, which is in contrast to the mechanism proposed by Cevc (7,24).

The results of the present study are also suggestive of a mechanism as a drug carrier system for three reasons:

1. The L-595/PEG-8-L (50/50) elastic vesicle formulation was clearly better than the PEG-8-L micelle solution. The present study showed that the elastic vesicles enhanced the rotigotine flux by a factor of 5 as compared to the PEG-8-L micelle solution. From this we can deduce that the improved drug transport by the elastic vesicles is not, or at least not solely, caused by the penetration-enhancing properties of the micelle-forming surfactant PEG-8-L.

2. Coapplication significantly enhanced the drug transport, whereas pretreatment showed no difference from the buffer control. This is in agreement with two previous studies investigating the effect of elastic vesicle coapplication and pretreatment on the transdermal flux of estradiol and per-

golide (10,15). From the above it can be concluded that the optimal drug transport can be achieved only when drug molecules are applied together with the elastic vesicles. This also suggests that a penetration-enhancing process is not the only—or not the most predominant—mechanism responsible for the increased drug transport after elastic vesicle treatment.

3. Higher entrapment efficiencies resulted in higher drug transport. From the results of this study it is evident that a high entrapment efficiency is essential in order to obtain the maximal drug transport enhancement effect. We can subsequently deduce that it is not sufficient to simply apply drug molecules together with the elastic vesicles (see point 2), but it is also necessary that these drug molecules are attached to the vesicles themselves.

In conclusion, our results have shown that (a) a penetration-enhancing effect of the individual surfactant components is not the main or the only mechanism of action for the elastic vesicles, and that (b) it is essential to apply drug molecules together with the vesicles as well as attached to the vesicles. It can, therefore, be suggested that the mechanism of action of the L-595/PEG-8-L elastic vesicles is most likely that of a drug carrier system rather than a penetration-enhancing process. This is in good agreement with a mechanism of action that was recently described by Honeywell-Nguyen *et al.*, who proposed that L-595/PEG-8-L elastic vesicles facilitate drug transport by a fast partitioning into the SC, thereby carrying vesicle-bound drug molecules into the skin (15).

CONCLUSIONS

This study has shown that elastic vesicles are significantly better than rigid vesicles, micelles, and buffer solutions in the enhancement of drug transport across human skin. A penetration-enhancing effect is not the main or the only mechanism of action of the elastic vesicles. However, it is likely that the elastic vesicles act as drug carrier systems. Hence, in order to obtain the highest drug transport, vesicle coapplication with a high drug entrapment efficiency is essential. The drug entrapment efficiency is strongly dependent on the pH of the drug-vesicular system. A high entrapment efficiency can therefore be achieved by choosing an optimal pH.

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