Effect of Elastic Liquid-State Vesicle on Apomorphine Iontophoresis Transport through Human Skin *In Vitro*

Gai Ling Li,[1] Meindert Danhof,[2] and Joke A. Bouwstra[1,3]

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INTRODUCTION

A major challenge in transdermal drug delivery is to overcome the skin inherent barrier. There is evidence that the primary barrier to transdermal diffusion is the stratum corneum (SC), which is the thin outmost layer of the skin. SC is composed of a regular array of protein rich cells embedded in a multilamellar lipid domain (1). The lipoid domains are the integral components of the transport barrier.

Many approaches, such as iontophoresis (2) or the application of chemical enhancers (3,4) have been suggested to enhance the penetration of drugs. Recently, it was proposed to use nonionic surfactant vesicles as drug carriers, often referred to as niosomes. These vesicles have several advantages over classic liposomes, in the sense of better chemical stability, ease of preparation, and low cost (5). The most recent development is the design of elastic liquid-state vesicles, which differ from conventional niosomes and liposomes by a characteristic fluid membrane and high elasticity (6). Studies with human stratum corneum *in vitro* and hairless mouse *in vivo* have shown that pretreatment of the skin with polyoxyethylene ester PEG-8-L containing elastic vesicles in a nonocclusive way modifies the ultrastructure in deeper layers of stratum corneum compared to rigid vesicles. Consequently an enhanced passive penetration of ^3H_2O was observed (7).

Apomorphine is a D_1 and D_2 dopamine receptor agonist, which has been proven to be very effective in the treatment of Parkinson's disease, particularly in the management of random response-fluctuations. However, due to its inherent instability, negligible oral biovailability, and short elimination half life, it cannot be administered orally (8,9). Recently in our group we focused on how to design a controlled delivery system for apomorphine by transdermal iontophoresis (10, 11). Application of a conventional iontophoretic system, however, resulted in maximum plasma levels in a lower therapeutical range. Therefore, it is important to increase the apomorphine delivery further. In the present study, we investigated whether treatment with elastic vesicles can be used to enhance apomorphine iontophoretic delivery. To this end, an elastic liquid-state vesicle suspension was prepared from polyoxyethylene ester PEG-8-L, sucrose-ester L595, and cholesterol sulfate at a molar ratio of 70: 30: 5, respectively. Its effects on the iontophoretic transport of apomorphine were examined in human SC and freshly dermatomed human epidermis *in vitro.*

MATERIALS AND METHODS

Materials

The polyoxyethylene ester PEG-8-L was a gift from Lipo Chemicals (Paterson, NJ). The sucrose-ester L595 was a kind gift from Mitsubishi Kasei (Tokyo, Japan). Cholesterol sulfate was purchased from Sigma Chemicals (Hilversum, The Netherlands). R-apomorphine hydrochloride was obtained from OPG (Utrecht, The Netherlands). Purity was tested by high performance chromatography (HPLC) on a chiral column and found to be >99%. Silver and silver chloride were obtained from Aldrich (Bornem, Belgium) and were more than 99.99% pure. HPLC grade acetonitrile (Rathburn, Walkerburn, Scotland) was used as a solvent in the HPLC analysis. All other chemicals used were of analytical grade. Millipore water (resistivity $\geq$ 18 MΩ) was used to prepare all solutions.

Preparation of Elastic Vesicle Suspension

The elastic vesicle suspension was prepared by a modification of the sonication method described by Baillie *et al.* (12). Briefly, the PEG-8-L, sucrose-ester L595, and cholesterol sulfate were solubilized in chloroform/methanol (3:1 v/v). The solvent was evaporated overnight in a vacuum centrifuge, and the remaining surfactant film was hydrated with phospate buffered saline (PBS). The final formulation contained 5% (w/w) of total lipids. Furthermore, the suspension was sonicated for 15 s at room temperature. The sonicator used was a Branson Sonifier 250 (Branson Ultrasonics Corp., Danbury, CT) with a 1/8 inch microtip at 60 watt energy output. The vesicle suspension was examined for size distribution and stability at room temperature during one week. The Dynamic Light Scattering determined the z-average of the vesicle.

Preparation of Epidermis and SC

Human abdomen skin was obtained after cosmetic surgery and processed on the same day. To avoid interference with sebaceous lipids or contamination of subcutaneous fat at the skin surface, the skin was carefully wiped with 70% ethanol. Extraction of skin lipids and penetration enhancement by ethanol is minimal during this cleaning procedure (13). Subcutaneous fat was removed after which the skin was dermatomed (Padgett Dermatome, Kansas City, KS), and SC was further obtained by the splitting technique described in detail

[1] Department of Pharmaceutical Technology, Leiden Amsterdam Center for Drug Research, University of Leiden, Leiden, The Netherlands.

[2] Department of Pharmacology, Leiden Amsterdam Center for Drug Research, University of Leiden, Leiden, The Netherlands.

[3] To whom correspondence should be addressed at Department of Pharmaceutical Technology, Leiden Amsterdam Center for Drug Research, University of Leiden, P.O. Box 9502, 2300 RA, Leiden, The Netherlands. (e-mail: Bouwstra@chem.leidenuniv.nl)

ABBREVIATIONS: SC, stratum corneum; PEG-8-L, polyoxyethylene ester PEG-8-L; L595, sucrose-ester L595; EM, electron microscopy; TPE, two-photon electroscope.

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previously (10). Dermatomed epidermis with 200–250 μm thickness was also used for the *in vitro* penetration studies.

In Vitro Iontophoresis Study

Prior to iontophoresis the SC/epidermis was treated non-occlusively for 3 h with 40 μl/cm^2 of vesicle suspension, or of PBS buffer as a control. Afterwards skin samples were transferred to the iontophoresis setup in which Ag (anode) and Ag/AgCl (cathode) were used as working electrodes. All diffusion experiments were carried out in three chamber continuous flow-through diffusion cells. The anodal chamber was filled with 15 mM citrate buffered apomorphine hydrochloride solution (5 mM citrate buffer, 8g/l NaCl, 1g/l sodium meta bisulphite, pH = 5.0). The cathodal chamber was filled with PBS pH = 7.4. The intermediate chamber was continuously perfused using a peristaltic pump with PBS buffer at 7 ml/h. Hourly samples were collected with a fraction collector. During the experiments both anodal and cathodal chambers were magnetically stirred at 375 rpm. Precautions were taken to prevent oxidation of apomorphine by using N_2 gas, keeping the system in dark, and adding 200 μl anti-oxidant solution (0.05% EDTA, 0.5% sodium meta bisulphite dissolved in 25% H_3PO_4) in the collecting tubes. The total course of the diffusion experiment included 3 stages: 6 h passive, 9 h iontophoresis at a constant current density of 500 μA/cm^2, and 5 h post-iontophoresis passive diffusion.

The samples were assayed by HPLC. The method utilized a nucleosil 100, 5 μm C18 column (200 ×4.6 mm I.D.) (YMC, Morris Planes, NJ) and a mobile phase of acetonitrile/aqueous phase (0.1 M NaH_2PO_4, 20 mg/ml 1-octanesulfonic acid, and 10 mg/ml EDTA, pH 3) at a ratio of 25:75 (v/v). The flow rate was 0.75 ml/min. Detection was by a fluorescence detector with the excitation wavelength set at 280 nm and emission wavelength at 460 nm.

Data Analysis

The cumulative amount of apomorphine permeated per unit skin area was plotted against time, the slope of the linear portion of the plot was estimated as the steady-state flux, and the time when cumulative flux reached zero as the lag time. The enhancement ratio was calculated by iontophoretic steady-state flux across vesicle treated skin divided by that across PBS-treated skin. Statistical comparisons were made using Student's *t* test. The probability value of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSIONS

In Fig. 1, the size of PEG-8-L/L595/Cholesterol sulfate vesicle formulation at a molar ratio of 70: 30: 5, is plotted as function of time. On day 1 the average size of the vesicles is 164.2 ± 2.6nm. Over the time course of 5 days the mean size of the vesicles did not increase significantly. These results are consistent with the previous observation (7). Furthermore, it is known that this vesicle suspension consists of perforated vesicles and threadlike micelles (7).

Figure 2 shows the permeation profiles of apomorphine across human SC treated with either elastic vesicle suspension or PBS during 6 h passive diffusion, 9 h iontophoresis diffusion, and 5 h post-iontophoresis passive diffusion. Similar permeation profiles of apomorphine were also observed with

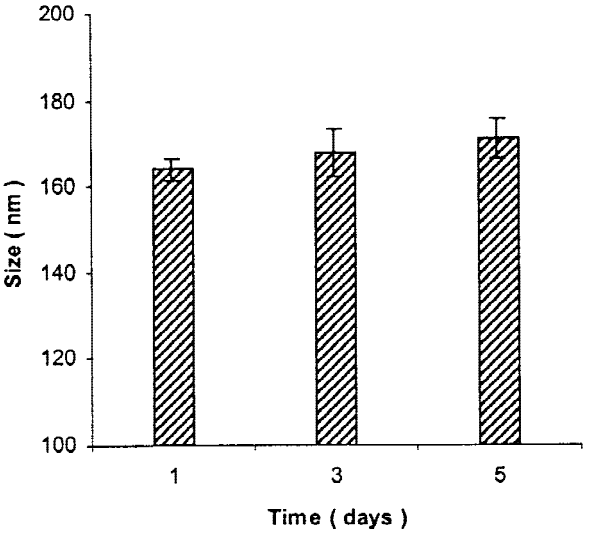

Fig. 1. Time course of the stability of elastic vesicle composed of PEG-8-L/L595/CSO_4 at the molar ratio of 70: 30: 5. Data are presented as mean ± SD (n = 3).

freshly dermatomed epidermis (not shown). As illustrated in Fig. 2, during 6 h pre-iontophoresis passive diffusion, the amount of apomorphine in the receptor chamber was below the detection limit of the HPLC method. After switching on the current, the apomorphine flux was dramatically increased and reached steady state within a short period. Following switching off of the current the flux decreased to a final post-iontophoretic plateau. Table I summarizes the calculated steady-state fluxes, enhancement ratios, and lag time during 9 h iontophoresis and 5 h post-iontophoresis passive fluxes. No degradation products and metabolites of apomorphine were present in either the donor or the acceptor phase.

Compared with the control (PBS), the iontophoretic transport of apomorphine was substantially enhanced by a factor of about 1.4 ($p < 0.01$) following the treatment with the

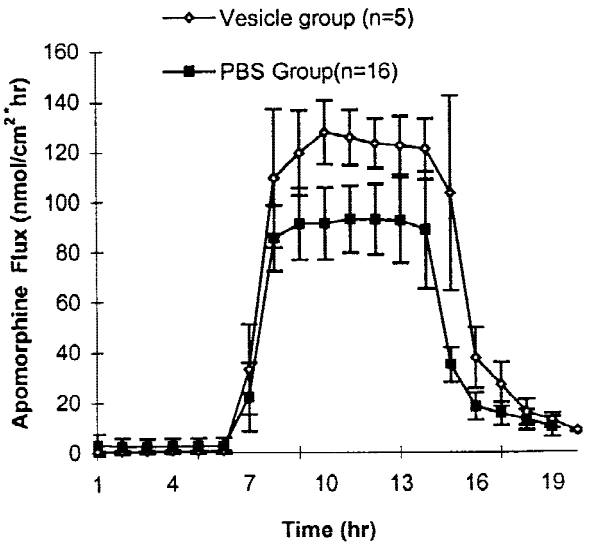

Fig. 2. Permeation profile of apomorphine across human SC during 6 h passive diffusion, 9 h iontophoresis at current density of 500 μA/cm^2, and 5 h post-iontophoresis passive diffusion. (■) Control; (◇) pretreatment with elastic vesicle.

Table I. Effect of Elastic Vesicle of PEG-8-L/L595/CSO_4 on Iontophoretic Delivery of Apomorphine through Human SC an Epidermis

Skin type		Iontophoretic steady-state flux (nmol/cm^2*hr)	Lag time (min)	Enhancement ratio	Post-iontophoretic passive flux (nmol/cm^2*hr)	Replicates
SC	Vesicle treated	123.8 ± 12.4*	51	1.35	7.9 ± 0.7	5
	Control	91.9 ± 16.0	48		8.2 ± 1.7	16
Epidermis	Vesicle treated	100.7 ± 8.3*	151	1.48	45.4 ± 8.9	7
	Control	67.9 ± 8.2	161		37.2 ± 9.1	6

Note: *Indicates statistically significant ($p < 0.01$) than the respective PBS control.

elastic vesicle suspension in both cases of diffusion through SC and human epidermis, whereas the corresponding lag time of iontophoresis and post-iontophoresis passive fluxes remained almost unchanged. Because vesicle pretreatment did not enhance the apomorphine passive transport prior to iontophoresis, a synergistic effect was observed after combining elastic vesicle pretreatment with iontophoresis. Further comparing SC and epidermis, significant differences were observed with respect to the iontophoretic lag time, steady-state flux during iontophoresis, and post-iontophoresis passive diffusion (see Table I). Specifically, the iontophoretic steady-state flux through epidermis was significantly lower by about 20% compared to the flux through SC, but the lag time was increased 3 times longer, and the post-iontophoresis passive flux was approximately 5-folds larger. These differences may be attributed to either one of the following issues. 1) The drug may bind to the tissue/protein in the viable skin; 2) The higher pH value in the viable skin will partly neutralize the drug, which makes iontophoresis less effective; 3) *In vitro* in the dermis layer no clearance will be observed in contrast to the *in vivo* situation.

From our results, it can be concluded that PEG-8-L elastic vesicle treatment in combination with iontophoresis provides an additional driving force to maintain and control the target flux of apomorphine. However, the mechanism of this effect remains a subject that warrants further investigation. In the literature (14,15), several mechanisms are proposed for the synergistic effect of iontophoresis and chemical enhancers, such as reduced skin impedance, increased enhancer deposition, and reduced size selectivity. Figure 3 shows that treatment with elastic vesicle suspension considerably diminished the steady-state resistance of human epidermis compared to the control. This implies that changes in the electric property of skin may be, at least partly, associated with the enhancing effect of the elastic vesicle. The previous study using electron microscopy (EM) and two-photon electroscope (TPE) technique (16) has shown that the elastic vesicle treatment induced strong ultrastructural changes mainly in large intercellular lipid regions within SC. More interestingly, a threadlike channel formation within intercellular spaces was visualized. These results indicate that by disrupting the skin lipid lamellar organization, penetration pathways might be created or modified through which apomorphine molecules can diffuse with less resistance. However, no clear evidence was obtained for intact permeation of the vesicles into the stratum corneum (16).

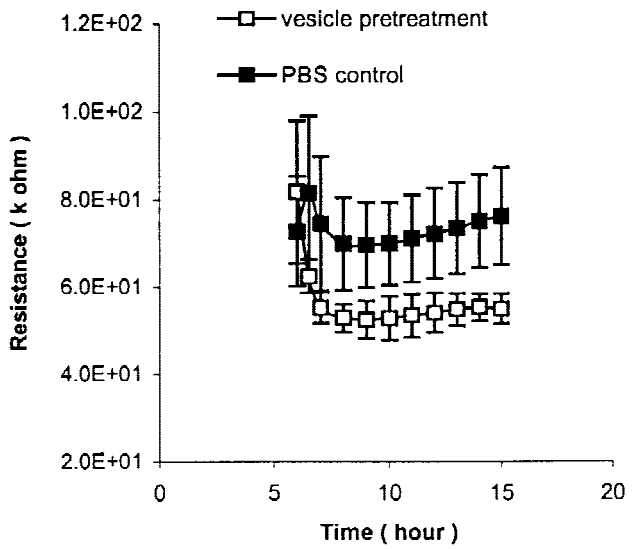

Fig. 3. Resistance of pretreated human epidermis as a function of time during 9 h iontophoresis at a current density of 500 $\mu A/cm^2$. Current is switched on at t = 6 h, and switched off at t = 15 h.

In summary, the present study identifies the synergistic effect of treatment with the elastic liquid-state vesicle suspension as a new approach to enhance and control the iontophoretic delivery of apomorphine through the skin. However, the magnitude of this synergy is moderate. Further optimization of vesicle composition, applied amount and charge, will be expected to improve the potential of this strategy. Thus, the utilization of elastic liquid-state vesicles may be of practical significance and essential in developing an effective transdermal iontophoresis device.

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